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(21) International Application Number: PCT/US99/12777 (22) International Filing Date: 7 June 1999 (07.06.99) (30) Priority Data: 60/088,465                      8 June 1998 (08.06.98)                      US 60/093,068                      16 July 1998 (16.07.98)                      US 60/113,864                      24 December 1998 (24.12.98)                      US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications US                                      60/088,465 (CON) Filed on                                      8 June 1998 (08.06.98) US                                      60/093,068 (CON) Filed on                                      16 July 1998 (16.07.98) US                                      60/113,864 (CON) Filed on                                      24 December 1998 (24.12.98) (71) Applicant (for all designated States except US): ADVANCED MEDICINE, INC. [US/US]; 280 Utah Avenue, South San Francisco, CA 94080 (US).		(72) Inventors; and (75) Inventors/Applicants (for US only): JACOBSEN, John, R. [US/US]; 23 Cityview Way, San Francisco, CA 94131 (US). EASTMAN, Donna [US/US]; 37 Don Gabriel Way, Orinda, CA 94563 (US). GRIFFIN, John, H. [US/US]; 56 Walnut Avenue, Atherton, CA 94027 (US). (74) Agents: SWISS, Gerald, F. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(54) Title: NOVEL POTASSIUM CHANNEL DRUGS AND THEIR USES			
(57) Abstract			
<p>This invention relates to novel multibinding compounds that bind to potassium (K<sup>+</sup>) channels and modulate their activity. The compounds of this invention comprise 2-10 K<sup>+</sup> channel ligands covalently connected by a linker or linkers, wherein the ligands in their monovalent (i.e., unlinked) state bind to one or more types of K<sup>+</sup> channel. The manner of linking the ligands together is such that the multibinding agents thus formed demonstrate an increased biologic and/or therapeutic effect as compared to the same number of unlinked ligands made available for binding to the K<sup>+</sup> channel. The invention also relates to methods of using such compounds and to methods of preparing them. The compounds of this invention are particularly useful for treating diseases and conditions of mammals that are mediated by K<sup>+</sup> channels. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.</p>			

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## NOVEL POTASSIUM CHANNEL DRUGS AND THEIR USES

### CROSS-REFERENCE TO RELATED APPLICATIONS

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This application claims priority to U.S. Applications Serial Nos. 60/088,465, filed June 8, 1998; 60/093,068, filed July 16, 1998; and 60/113,864, filed December 24, 1998, the entire contents of which are incorporated herein by reference.

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### BACKGROUND

#### Field of the Invention

This invention relates to novel multibinding compounds that bind to potassium ( $K^+$ ) channels and modulate their activity. The compounds of this invention comprise 2-10  $K^+$  channel ligands covalently connected by a linker or linkers, wherein the ligands in their monovalent (i.e., unlinked) state bind to one or more types of  $K^+$  channel. The manner of linking the ligands together is such that the multibinding agents thus formed demonstrate an increased biologic and/or therapeutic effect as compared to the same number of unlinked ligands made available for binding to the  $K^+$  channel. The invention also relates to methods of using such compounds and to methods of preparing them.

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The compounds of this invention are particularly useful for treating diseases and conditions of mammals that are mediated by  $K^+$  channels. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

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15       The disclosure of each of the above publications is incorporated herein by reference in its entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety.

#### State of the Art

20       Voltage-regulated potassium channels mediate the flux of K<sup>+</sup> out of cells in response to changes in membrane potential.<sup>28</sup> Voltage-gated K<sup>+</sup> channels in the open state typically transition to an inactivated state, and must reacquire the ability to respond to an external stimulus during a recovery period. An inward rectifying voltage-regulated potassium channel in cardiac muscle is also activated by acetylcholine (i.e., it is gated by more than one type of  
25       stimulus).<sup>18</sup> A calcium-activated K<sup>+</sup> channel has been described.<sup>16</sup> Potassium channels serve a variety of important cellular functions, including excitability, setting and maintaining the resting potential, repolarizing action potentials, transmembrane transport, volume regulation, signal transduction, and so on.<sup>28</sup> They are implicated in a variety of pathophysiological disorders, including hypertension, cardiac arrhythmogenesis, insulin-dependent diabetes, non-



insulin dependent diabetes mellitus, diabetic neuropathy, seizures, tachycardia, ischemic heart disease, cardiac failure, angina, myocardial infarction, transplant rejection, autoimmune disease, sickle cell anemia, muscular dystrophy, gastrointestinal disease, mental disorder, sleep disorder, anxiety disorder, neurosis, alcoholism, inflammation, cerebrovascular ischemia, CNS diseases, epilepsy, Parkinson's disease, asthma, incontinence, urinary dysfunction, micturition disorder, irritable bowel syndrome, restenosis, subarachnoid hemorrhage, Alzheimers disease, and they mediate the transmission of pain impulses by peripheral nerves.<sup>45</sup>

Figure 1 illustrates in cross-sectional view the transmembrane domain/subunit organization of various transporter molecules, as it is presently understood by those working in the field of transport physiology. It should be understood that, for purposes of simplification, other subunits that may be involved in or required for transporter activity have been omitted from the diagram.

Referring to Figure 1, voltage-gated ion channels and related proteins are tetrameric structures formed by the noncovalent association of individual subunits (1),(2), or by the interaction of homologous domains of a monomeric protein (3). The channels differ as well in the number of transmembrane segments per subunit or per domain. Inward-rectifier type  $K^+$  channels and  $P_{2x}$  purinergic channels have two transmembrane-segments in each subunit, Shaker-type  $K^+$  channels have six transmembrane segments per subunit and  $Na^+$  and  $Ca^{++}$  channels have six transmembrane segments per domain. Neurotransmitter-gated ion channels such as those shown in (4) are organized as pentamers, with each of the subunits having four transmembrane segments/domains. The activation gate for potassium channels has not been identified, although a trap door mechanism has been proposed.<sup>81,120</sup>

Potassium channels are structurally similar to, but smaller and simpler than, sodium and calcium ion channels,<sup>98</sup> with the  $K^+$  channel tetrameric structure being formed by four polypeptides.<sup>3</sup> However, potassium channels represent a diverse class of ion channels.<sup>18</sup>

Homotetramers can form, but there is evidence that heterotetramers may be functionally relevant *in vivo*.<sup>10</sup> The x-ray structure of a bacterial K<sup>+</sup> channel (which is homologous to mammalian K<sup>+</sup> channels) has been disclosed.<sup>21</sup> A prokaryotic K<sup>+</sup> channel was found to have the same structure as a eukaryotic K<sup>+</sup> channel.<sup>104</sup> The channel has an inverted teepee structure with a large hydrophobic cavity. The cavity (10Å) is centered in the channel on the cytoplasmic side, and appears to get larger upon channel opening.<sup>21,82,110,114</sup> Voltage-dependent cardiac potassium channel genes have been cloned as cDNAs.<sup>10,113,116</sup> Variability in the potassium channel genes may relate to disease conditions.<sup>14,48,50,70</sup>

Drug binding sites for tetraethylammonium, quinidine and 4-aminopyridine are found in the inner vestibule of the K<sup>+</sup> channel, and the amino acid side chains involved are localized in the S6 helix. Binding studies using mutagenesis show similarity to local anesthetic (LA) binding to sodium channels, although sodium channel inhibitors bind more deeply in the cavity.<sup>72,88</sup>

There appear to be two types of potassium channel inactivation, N-type and C-type, which can occur simultaneously in Shaker potassium channels. Both are partially coupled to activation and are usually voltage insensitive once activation is complete. N-type inactivation in Shaker B channels depends on a group of amino acids at the N-terminal that bind to the activated channel and occlude the intracellular mouth of the channel. No sequence similarity has been found among the N-termini of the N-type inactivating channels. N-type inactivation is voltage insensitive at positive potentials and competes with drug binding at the intracellular face of the channel. C-type inactivation, which is less understood, occurs by occlusion of the external mouth of the channel during sustained depolarization. C-type inactivation is voltage insensitive at potentials where activation is complete, but recovery from C-type inactivation is voltage sensitive. Both C- and N-type inactivation are coupled or partially coupled to activation, and both require similar degrees of activation to proceed.<sup>40</sup>

Not surprisingly, potassium channels are recognized as important targets for drug therapy. For example, potassium channels are targeted by certain antidiabetic, antihypertensive and antiarrhythmic drugs.

5 Potassium channel antagonists are used for treatment of arrhythmia. Antiarrhythmic agents are classified into four classes under the Vaughan Williams classification scheme: Class I (sodium channel blockers); Class II (beta-blockers); Class III (potassium channel blockers); and Class IV (calcium channel blockers). As shown in Table 1, an antiarrhythmic agent may have activity in several channels and/or with several receptors.<sup>89,92,101</sup> Newer drugs  
10 are more selective to specific K<sup>+</sup> channels, as shown in Table 2. Properties of some known K<sup>+</sup> channel blockers are given in Table 3. Table 5 sets forth the principal K<sup>+</sup> currents and some drugs that block them.<sup>45</sup> The majority of drugs in development are I<sub>Kr</sub> blockers.<sup>87,103,112</sup> Some agents appear to be cationic open-channel blockers.<sup>115,118,119</sup>

15 Combination therapy with two separate agents, e.g., a potassium channel opener with little or no effect on cardiac action potential and a Class III antiarrhythmic compound has been disclosed.<sup>75</sup>

The clinical shortcomings of drugs in current usage are considerable. Their most  
20 common adverse side effects include headache, hypotension, nausea, vomiting, dizziness, and the like. Other side effects may include photo sensitivity, corneal microdeposits, neuropathy, fatigue,<sup>35,39,41,56,61</sup> pneumonitis, hepatotoxicity, proarrhythmic effects,<sup>46</sup> thyroid abnormalities and bradycardia. Reverse use dependence, which may lead to torsades de pointes (an induced arrhythmia), is a major problem of most or all known Class III agents.<sup>42,43,47,96,117</sup> Further,  
25 there may be no survival benefit associated with the use of these agents.<sup>67</sup> With few exceptions, the currently used drugs have a short duration of action and must be administered frequently for sustained effects.

Thus, there continues to exist a need for novel compounds with greater tissue selectivity, increased efficacy, reduced side effects and a more favorable duration of action.

## SUMMARY OF THE INVENTION

5

This invention is directed to novel multibinding compounds that bind to K<sup>+</sup> channels in mammalian tissues and can be used to treat diseases and conditions mediated by such channels.

10

This invention is also directed to general synthetic methods for generating large libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties for potassium channels. The diverse multimeric compound libraries provided by this invention are synthesized by combining a linker or linkers with a ligand or ligands to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity and polarization. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

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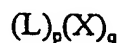
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This invention is also directed to libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties. These libraries are prepared via the methods described above and permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a ligand or a class of ligands targeting a potassium channel.

Accordingly, in one of its composition aspects, this invention is directed to a multibinding compound and salts thereof comprising 2 to 10 ligands which may be the same

or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel.

5 I: The multibinding compounds of this invention are preferably represented by Formula



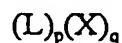
I

where each L is a ligand that may be the same or different at each occurrence; X is a linker that may be the same or different at each occurrence; *p* is an integer of from 2 to 10; and *q* is an integer of from 1 to 20; wherein each of said ligands comprises a ligand domain  
10 capable of binding to a K<sup>+</sup> channel. Preferably *q* is less than *p*.

Preferably, the binding of the multibinding compound to a K<sup>+</sup> channel or channels in a mammal modulates diseases and conditions mediated by the K<sup>+</sup> channel or channels.

15 In another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of one or more multibinding compounds (or pharmaceutically acceptable salts thereof) comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said  
20 ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel of a cell mediating mammalian diseases or conditions, thereby modulating the diseases or conditions.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a  
25 therapeutically effective amount of one or more multibinding compounds represented by Formula I:



I

or pharmaceutically acceptable salts thereof, where each L is a ligand that may be the same or different at each occurrence; X is a linker that may be the same or different at each

occurrence;  $p$  is an integer of from 2 to 10; and  $q$  is an integer of from 1 to 20; wherein each of said ligands comprises a ligand domain capable of binding to a  $K^+$  channel of a cell mediating mammalian diseases or conditions, thereby modulating the diseases or conditions. Preferably  $q$  is less than  $p$ .

5

In one of its method aspects, this invention is directed to a method for modulating the activity of a  $K^+$  channel in a biologic tissue, which method comprises contacting a tissue having a  $K^+$  channel with a multibinding compound (or pharmaceutically acceptable salts thereof) under conditions sufficient to produce a change in the activity of the channel in said tissue, wherein the multibinding compound comprises 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a  $K^+$  channel.

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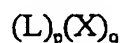
In another of its method aspects, this invention is directed to a method for treating a disease or condition in a mammal resulting from an activity of a  $K^+$  channel, which method comprises administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds (or pharmaceutically acceptable salts thereof) comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a  $K^+$  channel of a cell mediating mammalian diseases or conditions.

15

20

In yet another of its method aspects, this invention is directed to a method for treating a disease or condition in a mammal resulting from an activity of a  $K^+$  channel, which method comprises administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds represented by Formula I:

25



I

and pharmaceutically acceptable salts thereof, where each L is a ligand that may be the same or different at each occurrence; X is a linker that may be the same or different at each occurrence;  $p$  is an integer of from 2 to 10; and  $q$  is an integer of from 1 to 20; wherein each of said ligands comprises a ligand domain capable of binding to a  $K^+$  channel of a cell  
5 mediating mammalian diseases or conditions. Preferably  $q$  is less than  $p$ .

In a further aspect, this invention provides processes for preparing the multibinding agents of Formula I. This can be accomplished by combining  $p$  appropriately functionalized ligands with  $q$  complementary functionalized linkers under conditions where covalent bond  
10 formulation between the ligands and linkers occurs; alternatively, linking portions of  $p$  appropriately functionalized ligands to  $q$  complementary functionalized linkers and then completing the synthesis of the ligands in a subsequent step may be performed to prepare these compounds. Another method which may be used involves linking  $p$  appropriately functionalized ligands to portions of the linker(s) and then completing the synthesis of the  
15 linker(s) in a subsequent step. Coupling one or more of an appropriately functionalized ligand to a complementary functionalized linker, and subsequently coupling one or more additional ligands to said linker or linkers may be done to prepare the claimed compounds. Coupling as above wherein coupling of different appropriately functionalized linkers occurs simultaneously may also be used.

20

In one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at  
25 least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;  
5 and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

In another of its method aspects, this invention is directed to a method  
10 for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at  
15 least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;  
20 and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

25 The preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b). Sequential addition is preferred when a mixture of different ligands is employed to ensure heterodimeric or multimeric



compounds are prepared. Concurrent addition of the ligands occurs when at least a portion of the multimer compounds prepared are homomultimeric compounds.

5 The assay protocols recited in (d) can be conducted on the multimeric ligand compound library produced in (c) above, or preferably, each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

10 In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties for potassium channels, which library is prepared by the method comprising:

- (a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;
- (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and
- 15 (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

20

In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties for potassium channels, which library is prepared by the method comprising:

- 25 (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;
- (b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

5

In a preferred embodiment, the library of linkers employed in either the methods or the library aspects of this invention is selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and amphiphilic linkers. For example, in one embodiment, each of the linkers in the linker library may comprise linkers of different chain length and/or having different complementary reactive groups. Such linker lengths can preferably range from about 2 to 100Å.

10

In another preferred embodiment, the potassium channel ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands in order to provide for a range of orientations of said ligand on said multimeric ligand compounds. Such reactive functionality includes, by way of example, carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, and precursors thereof. It is understood, of course, that the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

15

20

In other embodiments, the multimeric ligand compound is homomeric (i.e., each of the ligands is the same, although it may be attached at different points) or heterodimeric (i.e., at least one of the ligands is different from the other ligands).

25

In addition to the combinatorial methods described herein, this invention provides for an iterative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor. Specifically, this method aspect is directed to a method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

Preferably, steps (e) and (f) are repeated at least two times, more preferably at from 2-50 times, even more preferably from 3 to 50 times, and still more preferably at least 5-50 times.

5

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a highly schematic illustration of the transmembrane organization of various cell membrane transporters.

10

Figure 2 illustrates a method for optimizing the linker geometry for presentation of ligands (filled circles) in bivalent compounds:

- A. phenyldiacetylene core structure
- B. cyclohexane dicarboxylic acid core structure

15

Figure 3 shows exemplary linker "core" structures.

Figure 4 illustrates examples of multi-binding compounds comprising (A) 2 ligands, (B) 3 ligands, (C) 4 ligands, and (D) >4 ligands attached in different formats to a linker.

20

Figure 5 illustrates the ligand amiodarone, which may be used in preparing multi-binding compounds. Potentially modifiable positions are indicated by arrows.

Figure 6 illustrates numerous reactive functional groups and the resulting bonds formed by reaction therebetween.

25

Figures 7 to 21 illustrate convenient methods for preparing the multibinding compounds of this invention. In each of these figures, the filled circles represent linkers, referred to in the written Examples as "Link".

## DETAILED DESCRIPTION OF THE INVENTION

Biological systems in general are controlled by molecular interactions between bioactive ligands and their receptors, in which the receptor "recognizes" a molecule or a portion thereof (i.e., a ligand domain) to produce a biological effect. The K<sup>+</sup> channels are considered to be pharmacological receptors: they possess specific binding sites for ligands having agonist and antagonist activities; the binding of ligands to such sites modulates K<sup>+</sup> flux through the channel; the channel properties (i.e., gating and ion selectivity) are regulatable. Accordingly, diseases or conditions that involve, or are mediated by, K<sup>+</sup> channels can be treated with pharmacologically active ligands that interact with such channels to initiate, modulate or abrogate transporter activity.

The interaction of a K<sup>+</sup> channel and a K<sup>+</sup> channel-binding ligand may be described in terms of "affinity" and "specificity". The "affinity" and "specificity" of any given ligand-K<sup>+</sup> channel interaction is dependent upon the complementarity of molecular binding surfaces and the energetic costs of complexation (i.e., the net difference in free energy between bound and free states). Affinity may be quantified by the equilibrium constant of complex formation, the ratio of on/off rate constants, and/or by the free energy of complex formation. Specificity relates to the difference in binding affinity of a ligand for different receptors.

The net free energy of interaction of such ligand with a K<sup>+</sup> channel is the difference between energetic gains (enthalpy gained through molecular complementarity and entropy gained through the hydrophobic effect) and energetic costs (enthalpy lost through decreased solvation and entropy lost through reduced translational, rotational and conformational degrees of freedom).

The compounds of this invention comprise 2 to 10 K<sup>+</sup> channel-binding ligands covalently linked together and capable of acting as multibinding agents. Without wishing to be bound by theory, the enhanced activity of these compounds is believed to arise at least in

part from their ability to bind in a multivalent manner with multiple ligand binding sites on a  $K^+$  channel or channels, which gives rise to a more favorable net free energy of binding.

Multivalent interactions differ from collections of individual monovalent (univalent) interactions by being capable of providing enhanced biologic and/or therapeutic effect.

5 Multivalent binding can amplify binding affinities and differences in binding affinities, resulting in enhanced binding specificity as well as affinity.

### Definitions

As used herein:

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The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-6 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, secondary butyl, tert-butyl, n-hexyl, n-octyl, n-decyl, n-dodecyl, 2-ethyldodecyl, 15 tetradecyl, and the like, unless otherwise indicated.

15

The term "substituted alkyl" refers to an alkyl group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, 20 amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and -NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or 25 different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

25

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, preferably 1-10 carbon

atoms, more preferably 1-6 carbon atoms. This term is exemplified by groups such as methylene ( $-\text{CH}_2-$ ), ethylene ( $-\text{CH}_2\text{CH}_2-$ ), the propylene isomers (e.g.,  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  and  $-\text{CH}(\text{CH}_3)\text{CH}_2-$ ) and the like.

5       The term "substituted alkylene" refers to: (1) An alkylene group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, 10       aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocycloxy, thioheterocycloxy, nitro, and  $-\text{NR}_a\text{R}_b$ , wherein  $\text{R}_a$  and  $\text{R}_b$  may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form 15       one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group; (2) An alkylene group as defined above that is interrupted by 1-20 atoms independently chosen from oxygen, sulfur and  $\text{NR}_a$ , where  $\text{R}_a$  is chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic, or groups 20       selected from carbonyl, carboxyester, carboxyamide and sulfonyl; and (3) An alkylene group as defined above that has both from 1 to 5 substituents as defined above and is also interrupted by 1-20 atoms as defined above. Examples of substituted alkylenes are chloromethylene ( $-\text{CH}(\text{Cl})-$ ), aminoethylene ( $-\text{CH}(\text{NH}_2)\text{CH}_2-$ ), 2-carboxypropylene isomers ( $-\text{CH}_2\text{CH}(\text{CO}_2\text{H})\text{CH}_2-$ ), ethoxyethyl ( $-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-$ ), ethylmethylaninoethyl 25       ( $-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_2-$ ), 1-ethoxy-2-(2-ethoxy-ethoxy)ethane ( $-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{OCH}_2\text{CH}_2-\text{OCH}_2\text{CH}_2-$ ), and the like.

The term "alkaryl" or "aralkyl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl in which alkylene and aryl are as defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

5 The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

10 The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

15 The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Examples of such groups are methylenemethoxy ( $-\text{CH}_2\text{OCH}_3$ ), ethylenemethoxy ( $-\text{CH}_2\text{CH}_2\text{OCH}_3$ ), n-propylene-iso-propoxy ( $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OCH}(\text{CH}_3)_2$ ), methylene-t-butoxy ( $-\text{CH}_2-\text{O}-\text{C}(\text{CH}_3)_3$ ) and the like.

20 The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein.

25 Preferred alkylthioalkoxy groups are alkylene-S-alkyl and include, by way of example, methylenethiomethoxy ( $-\text{CH}_2\text{SCH}_3$ ), ethylenethiomethoxy ( $-\text{CH}_2\text{CH}_2\text{SCH}_3$ ), n-propylene-iso-thiopropoxy ( $-\text{CH}_2\text{CH}_2\text{CH}_2\text{SCH}(\text{CH}_3)_2$ ), methylene-t-thiobutoxy ( $-\text{CH}_2\text{SC}(\text{CH}_3)_3$ ) and the like.



“Alkenyl” refers to a monoradical of a branched or unbranched unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 double bonds. This term is further exemplified by such radicals as vinyl, prop-2-enyl, pent-3-enyl, hex-5-enyl, 5-ethyldodec-3,6-dienyl, and the like.

The term “substituted alkenyl” refers to an alkenyl group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, aryloxy, thioaryloxy, heteroaryloxy, thioheteroaryloxy, heterocycloxy, thioheterocycloxy, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and, -NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

“Alkenylene” refers to a diradical of an unsaturated hydrocarbon, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 double bonds. This term is further exemplified by such radicals as 1,2-ethenyl, 1,3-prop-2-enyl, 1,5-pent-3-enyl, 1,4-hex-5-enyl, 5-ethyl-1,12-dodec-3,6-dienyl, and the like.

The term “substituted alkenylene” refers to an alkenylene group as defined above having from 1 to 5 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocycloxy, thioheterocycloxy, nitro, and NR<sup>a</sup>R<sup>b</sup>,

wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl,  
5 cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

"Alkynyl" refers to a monoradical of an unsaturated hydrocarbon, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms,  
10 and preferably having 1-6 triple bonds. This term is further exemplified by such radicals as acetylenyl, prop-2-ynyl, pent-3-ynyl, hex-5-ynyl, 5-ethyldodec-3,6-diynyl, and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, selected from the group consisting of alkoxy, substituted alkoxy,  
15 acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocycloxy, thioheterocycloxy, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, SO<sub>2</sub>-  
20 heterocyclic, NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

"Alkynylene" refers to a diradical of an unsaturated hydrocarbon radical, preferably  
25 having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 triple bonds. This term is further exemplified by such radicals as 1,3-prop-2-ynyl, 1,5-pent-3-ynyl, 1,4-hex-5-ynyl, 5-ethyl-1,12-dodec-3,6-diynyl, and the like.

5 The term "acyl" refers to the groups -CHO, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

10 The term "acylamino" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholine) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

15 The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyloxy" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

20 The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

25 The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl).

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, trihalomethyl, NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "arylene" refers to a diradical derived from aryl or substituted aryl as defined above, and is exemplified by 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "amino" refers to the group -NH<sub>2</sub>.

The term "substituted amino" refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" refers to the group "-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl", "-C(O)O-alkynyl" and "-C(O)O-substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl where alkynyl are as defined herein.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring or fused rings and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl,

substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

"Haloalkyl" refers to alkyl as defined above substituted by 1-4 halo groups as defined above, which may be the same or different, such as 3-fluorododecyl, 12,12,12-trifluorododecyl, 2-bromooctyl, -3-bromo-6-chloroheptyl, and the like.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, trihalomethyl,

mono- and di-alkylamino, mono- and  $\text{NR}^a\text{R}^b$ , wherein  $\text{R}^a$  and  $\text{R}^b$  may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

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The term "heteroaryloxy" refers to the group heteroaryl-O-.

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The term "heteroarylene" refers to the diradical group derived from heteroaryl or substituted heteroaryl as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylenes, 1,8-quinolinylenes, 1,4-benzofuranylene, 2,5-pyridinylenes, 1,3-morpholinylenes, 2,5-indolenyl, and the like.

15

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

20

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and  $\text{NR}^a\text{R}^b$ , wherein  $\text{R}^a$  and  $\text{R}^b$  may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Such heterocyclic groups can have a single ring or multiple condensed rings.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

A preferred class of heterocyclics include "crown compounds" which refers to a specific class of heterocyclic compounds having one or more repeating units of the formula  $[-(\text{CH}_2)_m\text{Y}-]$  where  $m$  is equal to or greater than 2, and  $\text{Y}$  at each separate occurrence can be O, N, S or P. Examples of crown compounds include, by way of example only,  $[-(\text{CH}_2)_3\text{NH}-]_3$ ,  $[-((\text{CH}_2)_2\text{-O})_4-((\text{CH}_2)_2\text{-NH})_2]$  and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term "heterocyclooxy" refers to the group heterocyclic-O-.

The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

The term "heterocyclene" refers to the diradical group derived from a heterocycle as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

The term "oxyacylamino" refers to the group  $-\text{OC}(\text{O})\text{NRR}$  where each  $\text{R}$  is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "thiol" refers to the group -SH.



The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

5       The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

10       The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

15       As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

20       "Alkyl optionally interrupted by 1-5 atoms chosen from O, S, or N" refers to alkyl as defined above in which the carbon chain is interrupted by O, S, or N. Within the scope are ethers, sulfides, and amines, for example 1-methoxydecyl, 1-pentyloxynonane, 1-(2-isopropoxyethoxy)-4-methylnonane, 1-(2-ethoxyethoxy)dodecyl, 2-(t-butoxy)heptyl, 1-pentylsulfanylnonane, nonylpentylamine, and the like.

25       "Heteroarylalkyl" refers to heteroaryl as defined above linked to alkyl as defined above, for example pyrid-2-ylmethyl, 8-quinolinypropyl, and the like.

      "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, optionally

substituted alkyl means that alkyl may or may not be substituted by those groups enumerated in the definition of substituted alkyl.

5 The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the multibinding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multibinding compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

10 Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl)  
15 amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines,  
20 disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl,  
25 substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group. *See, generally, T.W. Greene & P.G.M. Wuts, Protective Groups in Organic Synthesis, 2<sup>nd</sup> Ed., 1991, John Wiley and Sons, N.Y.*

The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidene, phenacyl, *t*-butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively

removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

Preferred removable amino blocking groups include conventional substituents such as  
5 t-butyloxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), fluorenylmethoxycarbonyl (Fmoc),  
allyloxycarbonyl (ALOC) and the like, which can be removed by conventional conditions  
compatible with the nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl,  
10 t-butyl etc. which can be removed by mild hydrolysis conditions compatible with the nature  
of the product.

As used herein, the terms "inert organic solvent" or "inert solvent" mean a solvent  
inert under the conditions of the reaction being described in conjunction therewith [including,  
15 for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide  
("DMF"), chloroform ("CHCl<sub>3</sub>"), methylene chloride (or dichloromethane or "CH<sub>2</sub>Cl<sub>2</sub>"),  
diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol,  
isopropanol, tert-butanol, dioxane, pyridine, and the like]. Unless specified to the contrary,  
the solvents used in the reactions of the present invention are inert solvents.

20 The term "K<sup>+</sup> channel" refers to a structure comprised of integral membrane proteins  
that functions to allow K<sup>+</sup> to equilibrate across a membrane according to its electrochemical  
gradient and at rates that are diffusion limited.

25 "Ligand" as used herein denotes a compound that is a binding partner for a K<sup>+</sup> channel  
receptor, and is bound thereto, for example, by complementarity. The specific region or  
regions of the ligand molecule that is recognized by the ligand binding site of a K<sup>+</sup> channel  
receptor is designated as the "ligand domain". A ligand may be either capable of binding to a

receptor by itself, or may require the presence of one or more non-ligand components for binding (e.g. ions, a lipid molecule, a solvent molecule, and the like).

Ligands useful in this invention comprise K<sup>+</sup> channel modulators such as quinidine,<sup>6,94</sup>  
5 glibenclamide, procaine, tetraethyl ammonium,<sup>20</sup> clofilium,<sup>102</sup> melperone,<sup>8</sup> pinacidil, WAY-  
123,398,<sup>91</sup> cromakalim,<sup>26</sup> propofol, thiopentone,<sup>32</sup> risotilide, almokalant,<sup>36</sup> bretylium,<sup>38</sup> N-  
acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356,<sup>45</sup> afinidine,<sup>49</sup>  
chromanol 293B,<sup>57</sup> L-768,673 and its analogs,<sup>53</sup> bethanidine,<sup>54</sup> disopyramide,<sup>23</sup>  
desethylamiodarone,<sup>1</sup> NE-10064,<sup>9,84</sup> artilide,<sup>11</sup> dofetilide,<sup>19,73,74,90,99</sup> E-4031,<sup>24,99,109</sup>  
10 sematilide,<sup>106,107</sup> ambasilide, azimilide,<sup>5,80,86,93,100,108</sup> tedisamil, dronedarone,<sup>79</sup> ibutilide,<sup>78,111</sup>  
sotalol,<sup>85</sup> benzodiazepine analogs<sup>76,77</sup> and amiodarone.<sup>55,65,83,95,105</sup> See Table 4 for structures of  
various potassium channel ligands.

While it is contemplated that many potassium channel ligands that are currently  
15 known can be used in the preparation of multibinding compounds of this invention (Table 2),  
it should be understood that portions of the ligand structure that are not essential for  
molecular recognition and binding activity (i.e., that are not part of the ligand domain) may be  
varied substantially, replaced with unrelated structures and, in some cases, omitted entirely  
without affecting the binding interaction. Accordingly, it should be understood that the term  
20 "ligand" is not intended to be limited to compounds known to be useful as K<sup>+</sup> channel  
receptor-binding compounds (e.g., known drugs), in that ligands that exhibit marginal activity  
or lack useful activity as monomers can be highly active as multibinding compounds, because  
of the biological benefit conferred by multivalency. The primary requirement for a ligand as  
defined herein is that it has a ligand domain, as defined above, which is available for binding  
25 to a recognition site on a K<sup>+</sup> channel.

For purposes of the present invention, the term "ligand" or "ligands" is intended to  
include the racemic ligands as well as the individual stereoisomers of the ligands, including  
pure enantiomers and non-racemic mixtures thereof. The scope of the invention as described

and claimed encompasses the racemic forms of the ligands as well as the individual enantiomers and non-racemic mixtures thereof.

5 The term "ligand binding site" as used herein denotes a site on a  $K^+$  channel receptor that recognizes a ligand domain and provides a binding partner for the ligand. The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example agonism, antagonism, modulation, or may maintain an ongoing biological event, and the like.

10 It should be recognized that the ligand binding sites of  $K^+$  channel receptors that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and intermolecular associations. For example,  $K^+$  channel ligand binding sites may be covalently joined in a single structure, noncovalently associated in one or more multimeric structures, embedded in a membrane or biopolymer matrix, and so on, and  
15 therefore have less translational and rotational freedom than if the same sites were present as monomers in solution.

The terms "agonism" and "antagonism" are well known in the art. As used herein, the term "agonist" refers to a ligand that when bound to a  $K^+$  channel stimulates its activity. The  
20 term "antagonist" refers to a ligand that when bound to a  $K^+$  channel inhibits its activity. Channel block or activation may result from allosteric effects of ligand binding to the channel rather than occupancy of the channel pore. These allosteric effects may produce changes in protein conformation that affect  $K^+$  binding sites, gating mechanisms and/or the pore region (i.e., ion permeation).

25 A potassium channel can exist in several modes: C (closed resting state);  $C^*$  (activated closed state); O (open state); and I (inactivated state).<sup>44</sup> The probability that a channel will exist in one of these four states changes with voltage. A given ligand may have

different binding affinities for different states, and be capable of producing agonist or antagonist activity.

5 The term "modulatory effect" is intended to refer to the ability of a ligand to change the activity of a  $K^+$  channel through binding to the channel.

"Multibinding agent" or "multibinding compound" refers herein to a compound that has from 2 to 10  $K^+$  channel ligands as defined herein (which may be the same or different) covalently bound to one or more linkers (which may be the same or different), and is capable of multivalency, as defined below.

10

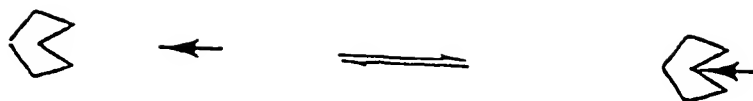
A multibinding compound provides an improved biologic and/or therapeutic effect compared to that of the same number of unlinked ligands available for binding to the ligand binding sites on a  $K^+$  channel or channels. Examples of improved "biologic and/or therapeutic effect" include increased ligand-receptor binding interactions (e.g., increased affinity, increased ability to elicit a functional change in the target, improved kinetics), increased selectivity for the target, increased potency, increased efficacy, decreased toxicity, increased therapeutic index, improved duration of action, improved bioavailability, improved pharmacokinetics, improved activity spectrum, and the like. The multibinding compounds of this invention will exhibit at least one, and preferably more than one, of the above-mentioned effects.

15

20

"Univalency" as used herein refers to a single binding interaction between one ligand with one ligand binding site as defined herein. It should be noted that a compound having multiple copies of a ligand (or ligands) exhibits univalency when only one ligand of that compound interacts with a ligand binding site. Examples of univalent interactions are depicted below.

25

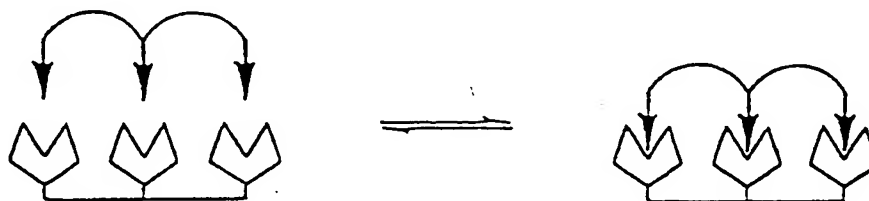


univalent interaction

5

"Multivalency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands, which may be the same or different, and two or more corresponding ligand binding sites, which may be the same or different. An example of trivalent binding is depicted below for illustrative purposes.

10



15

trivalent interaction

20

It should be understood that not all compounds that contain multiple copies of a ligand attached to a linker necessarily exhibit the phenomena of multivalency, i.e., that the biologic and/or therapeutic effect of the multibinding agent is greater than that of the same number of unlinked ligands made available for binding to the ligand binding sites. For multivalency to occur, the ligand domains of the ligands that are linked together must be presented to their cognate ligand binding sites by the linker or linkers in a specific manner in order to bring about the desired ligand-orienting result, and thus produce a multibinding interaction.

25

The term "library" refers to at least 3, preferably from  $10^2$  to  $10^9$  and more preferably from  $10^2$  to  $10^4$  multimeric compounds. Preferably, these compounds are prepared as a multiplicity of compounds in a single solution or reaction mixture which permits facile synthesis thereof. In one embodiment, the library of multimeric compounds can be directly assayed for multibinding properties. In another embodiment, each member of the library of



multimeric compounds is first isolated and, optionally, characterized. This member is then assayed for multibinding properties.

5 The term "collection" refers to a set of multimeric compounds which are prepared either sequentially or concurrently (e.g., combinatorially). The collection comprises at least 2 members; preferably from 2 to  $10^9$  members and still more preferably from 10 to  $10^4$  members.

10 The term "multimeric compound" refers to compounds comprising from 2 to 10 ligands covalently connected through at least one linker which compounds may or may not possess multibinding properties (as defined herein).

15 The term "pseudohalide" refers to functional groups which react in displacement reactions in a manner similar to a halogen. Such functional groups include, by way of example, mesyl, tosyl, azido and cyano groups.

The term "linker", identified where appropriate by the symbol X, refers to a group or groups that covalently links from 2 to 10 ligands (as defined above) in a manner that provides a compound capable of multivalency. The linker is a ligand-orienting entity that permits  
20 attachment of multiple copies of a ligand (which may be the same or different) thereto.

The term "linker" includes everything that is not considered to be part of the ligand, e.g., ancillary groups such as solubilizing groups, lipophilic groups, groups that alter pharmacodynamics or pharmacokinetics, groups that modify the diffusability of the  
25 multibinding compound, spacers that attach the ligand to the linker, groups that aid the ligand-orienting function of the linker, for example, by imparting flexibility or rigidity to the linker as a whole, or to a portion thereof, and so on. The term "linker" does not, however, cover solid inert supports such as beads, glass particles, rods, and the like, but it is to be understood that the multibinding compounds of this invention can be attached to a solid

understood that the multibinding compounds of this invention can be attached to a solid support if desired, for example, for use in separation and purification processes and for similar applications.

5           The extent to which the previously discussed enhanced activity of multibinding compounds is realized in this invention depends upon the efficiency with which the linker or linkers that joins the ligands presents them to their array of ligand binding sites. Beyond presenting these ligands for multivalent interactions with ligand binding sites, the linker spatially constrains these interactions to occur within dimensions defined by the linker.

10           The linkers used in this invention are selected to allow multivalent binding of ligands to any desired ligand binding sites of a  $K^+$  channel, whether such sites are located within the cell membrane, interiorly (e.g., within a channel/translocation pore), both interiorly and on the periphery of a channel, at the boundary region between the lipid bilayer and the channel, or at  
15           any intermediate position thereof. The preferred linker length will vary depending on the distance between adjacent ligand binding sites, and the geometry, flexibility and composition of the linker. The length of the linker will preferably be in the range of about 2Å to about 100Å, more preferably from about 2Å to about 50Å and even more preferably from about 5Å to about 20Å.

20           The ligands are covalently attached to the linker or linkers using conventional chemical techniques. The reaction chemistries resulting in such linkage are well known in the art and involve the use of reactive functional groups present on the linker and ligand. Preferably, the reactive functional groups on the linker are selected relative to the functional  
25           groups available on the ligand for coupling, or which can be introduced onto the ligand for this purpose. Again, such reactive functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable well-known activating agents results in formation of an amide bond covalently linking the ligand to the linker;

reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker. The table below and Figure 6 illustrate numerous reactive functional groups and the resulting bonds formed by reaction therebetween. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as J. March, *Advanced Organic Chemistry*, 4<sup>th</sup> Ed., (Wiley-Interscience, N.Y., 1992).

Complementary Binding Chemistries

First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	$\beta$ -hydroxyamine
sulfonyl halide	amine	sulfonamide
carboxyl	amine	amide
hydroxyl	alkyl/aryl halide	ether
amine	alkyl halide	substituted amine

The linker is attached to the ligand at a position that retains ligand domain-ligand binding site interaction and specifically which permits the ligand domain of the ligand to orient itself to bind to the ligand binding site. Such positions and synthetic protocols for linkage are well known in the art. The term linker embraces everything that is not considered to be part of the ligand.

The relative orientation in which the ligand domains are displayed depends both on the particular point or points of attachment of the ligands to the linker, and on the framework geometry. The determination of where acceptable substitutions can be made on a ligand is

typically based on prior knowledge of structure-activity relationships of the ligand and/or congeners and/or structural information about ligand-receptor complexes (e.g., X-ray crystallography, NMR, and the like). Such positions and synthetic protocols for linkage are well known in the art and can be determined by those with ordinary skill in the art (*see, e.g.,*

5 **METHODS OF PREPARATION**, Examples 1-29 and Figures 7 to 21. Following attachment of a ligand to the linker or linkers, or to a significant portion thereof (e.g., 2-10 atoms of linker), the linker-ligand conjugate may be tested for retention of activity in a relevant assay system (*see Utility and Testing* below for representative assays).

10 At present, it is preferred that the multibinding compound is a bivalent compound in which two ligands are covalently linked, or a trivalent compound, in which three ligands are covalently linked. Linker design is further discussed under **METHODS OF PREPARATION**.

15 "Potency" as used herein refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its receptor. In some cases, the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multibinding agent and the aggregate of its unlinked ligand, the dose-response curve of each  
20 is determined under identical test conditions (e.g., in an *in vitro* or *in vivo* assay, in an appropriate animal model (such as a human patient)). The finding that the multibinding agent produces an equivalent biologic or therapeutic effect at a lower concentration than the aggregate unlinked ligand (e.g., on a per weight, per mole or per ligand basis) is indicative of enhanced potency.

25 "Selectivity" or "specificity" is a measure of the binding preferences of a ligand for different receptors. The selectivity of a ligand with respect to its target receptor relative to another receptor is given by the ratio of the respective values of  $K_d$  (i.e., the dissociation constants for each ligand-receptor complex) or, in cases where a biological effect is observed

below the  $K_d$ , the ratio of the respective  $EC_{50}$ s or  $IC_{50}$ s (i.e., the concentrations that produce 50% of the maximum response for the ligand interacting with the two distinct receptors).

5 The term "treatment" refers to any treatment of a disease or condition in a mammal, particularly a human, and includes:

- (i) preventing the disease or condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the condition and, accordingly, the treatment constitutes prophylactic treatment for the pathologic condition;
- (ii) inhibiting the disease or condition, i.e., arresting its development;
- 10 (iii) relieving the disease or condition, i.e., causing regression of the disease or condition; or
- (iv) relieving the symptoms resulting from the disease or condition without addressing the underlying disease or condition, e.g., relieving symptoms of angina pectoris and other conditions of ischemia but not an underlying cause such as, for example,
- 15 atherosclerotic disease or hypertension.

The phrase "disease or condition which is modulated by treatment with a multibinding  $K^+$  channel ligand" covers all disease states and/or conditions that are generally acknowledged in the art to be usefully treated with a ligand for a  $K^+$  channel in general, and  
20 those disease states and/or conditions that have been found to be usefully treated by a specific multibinding compound of our invention, i.e., the compounds of Formula I. Such disease states include, by way of example only, hypertension, cerebral ischemia, cardiac arrhythmias (particularly, arrhythmias resulting from potassium-related changes in membrane potential and conduction), cardiac hypertrophy due to systolic or diastolic overload, congestive heart  
25 failure, and the like.

The term "therapeutically effective amount" refers to that amount of multibinding compound that is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending

upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

5           The term "pharmaceutically acceptable excipient" is intended to include vehicles and carriers capable of being coadministered with a multibinding compound to facilitate the performance of its intended function. The use of such media for pharmaceutically active substances is well known in the art. Examples of such vehicles and carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. Any other conventional  
10 carrier suitable for use with the multibinding compounds also falls within the scope of the present invention.

## METHODS OF PREPARATION

### 15    Linkers

          The linker or linkers, when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multibinding compound. The biological activity of the multibinding K<sup>+</sup> channel compound is highly sensitive to the geometry, composition, size, length, flexibility or rigidity, the presence or absence of anionic  
20 or cationic charge, the relative hydrophobicity/hydrophilicity, and similar properties of the linker. Accordingly, the linker is preferably chosen to maximize the biological activity of the compound. The linker may be biologically "neutral," i.e., not itself contribute any additional biological activity to the multibinding compound, or it may be chosen to further enhance the biological activity of the compound. In general, the linker may be chosen from any organic  
25 molecule construct that orients two or more ligands for binding to the receptors to permit multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand-orienting result, and thus produce a multibinding compound.

For example, different orientations of ligands can be achieved by varying the geometry of the framework (linker) by use of mono- or polycyclic groups, such as aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). The optimal geometry and composition of frameworks (linkers) used in the multibinding compounds of this invention are based upon the properties of their intended receptors. For example, it is preferred to use rigid cyclic groups (e.g., aryl, heteroaryl), or non-rigid cyclic groups (e.g., cycloalkyl or crown groups) to reduce conformational entropy when such may be necessary to achieve energetically coupled binding.

Different hydrophobic/hydrophilic characteristics of the linker as well as the presence or absence of charged moieties can readily be controlled by the skilled artisan. For example, the hydrophobic nature of a linker derived from hexamethylene diamine ( $\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2$ ) or related polyamines can be modified to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group such as found in the commercially available "Jeffamines" (class of surfactants).

Different frameworks can be designed to provide preferred orientations of the ligands. The identification of an appropriate framework geometry for ligand domain presentation is an important first step in the construction of a multi binding agent with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process. Figure 2 illustrates a useful strategy for determining an optimal framework display orientation for ligand domains and can be used for preparing the bivalent compounds of this invention. Various alternative strategies known to those skilled in the art of molecular design can be substituted for the one described here.

As shown in Figure 2, the ligands (shown as filled circles) are attached to a central core structure such as phenyldiacetylene (Panel A) or cyclohexane dicarboxylic acid (Panel B). The ligands are spaced apart from the core by an attaching moiety of variable lengths *m*

and *n*. If the ligand possesses multiple attachment sites (see discussion below), the orientation of the ligand on the attaching moiety may be varied as well. The positions of the display vectors around the central core structures are varied, thereby generating a collection of compounds. Assay of each of the individual compounds of a collection generated as  
5 described will lead to a subset of compounds with the desired enhanced activities (e.g., potency, selectivity). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will suggest a framework orientation that favors the properties desired.

The process may require the use of multiple copies of the same central core structure  
10 or combinations of different types of display cores. It is to be noted that core structures other than those shown here can be used for determining the optimal framework display orientation of the ligands. The above-described technique can be extended to trivalent compounds and compounds of higher-order valency.

A wide variety of linkers is commercially available (Chem Sources USA and Chem  
15 Sources International; the ACD electronic database; and Chemical Abstracts). Many of the linkers that are suitable for use in this invention fall into this category. Others can be readily synthesized by methods known in the art, and as described below. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like.  
20 Specific examples are peptides or polyamides, hydrocarbons, aromatics, heterocyclics, ethers, lipids, cationic or anionic groups, or a combination thereof.

Examples are given below and in Figure 3, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true  
25 spirit and scope of the invention. For example, properties of the linker can be modified by the addition or insertion of ancillary groups into the linker, for example, to change the solubility of the multibinding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto the linker enhances the



hydrophilicity and water solubility of the multibinding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further, PEG may decrease antigenicity and potentially enhances the overall rigidity of the linker.

5

Ancillary groups that enhance the water solubility/hydrophilicity of the linker, and accordingly, the resulting multibinding compounds, are useful in practicing this invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, small repeating units of ethylene glycols, alcohols, polyols, (e.g., glycerin, glycerol  
10 propoxylate, saccharides, including mono-, oligosaccharides, etc.) carboxylates (e.g., small repeating units of glutamic acid, acrylic acid, etc.), amines (e.g., tetraethylenepentamine), and the like to enhance the water solubility and/or hydrophilicity of the multibinding compounds of this invention. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyether. In particularly preferred embodiments, the  
15 ancillary group will contain a small number of repeating ethylene oxide ( $-\text{CH}_2\text{CH}_2\text{O}-$ ) units.

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the compounds of Formula I is also within the scope of this invention. Lipophilic groups useful with the linkers of this invention  
20 include, but are not limited to, lower alkyl, aromatic groups and polycyclic aromatic groups. The aromatic groups may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. As used herein the term "aromatic groups" incorporates both aromatic hydrocarbons and heterocyclic aromatics. Other lipophilic groups useful with the linkers of this invention include fatty acid  
25 derivatives which may or may not form micelles in aqueous medium and other specific lipophilic groups which modulate interactions between the multibinding compound and biological membranes.

Also within the scope of this invention is the use of ancillary groups which result in the compound of Formula I being incorporated into a vesicle, such as a liposome, or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer or micelle such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine and dilinoleoylphosphatidylcholine. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families, are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the linker can be manipulated by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker, or bonds between the linker and the ancillary group(s), or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational freedom is restrained by the presence of rings and/or  $\pi$ -bonds, for example, aryl, heteroaryl and heterocyclic groups. Other groups which can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the linker

into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other, which is inversely related to the square of the distance between the groups, will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups.

5 Further, ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker in a conformation which allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, protected groups that bear a latent charge which is  
10 unmasked, following addition to the linker, by deprotection, a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art, is within the scope of this invention.

Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal  
15 ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon  $\pi$ -bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

20 In preferred embodiments, rigidity (entropic control) is imparted by the presence of alicyclic (e.g., cycloalkyl), aromatic and heterocyclic groups. In other preferred embodiments, this comprises one or more six-membered rings. In still further preferred embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl, or a  
25 macrocyclic ring such as, for example, a crown compound.

In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art.

Eliminating or reducing antigenicity of the multibinding compounds described herein is also within the scope of this invention. In certain cases, the antigenicity of a multibinding compound may be eliminated or reduced by use of groups such as, for example, poly(ethylene glycol).

5

#### The Compounds of Formula I

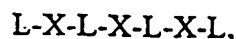
As explained above, the multibinding compounds described herein comprise 2-10 ligands attached covalently to a linker that links the ligands in a manner that allows their multivalent binding to ligand binding sites of  $K^+$  channels. The linker spatially constrains these interactions to occur within dimensions defined by the linker. This and other factors increases the biologic and/or therapeutic effect of the multibinding compound as compared to the same number of ligands used in monobinding form.

The compounds of this invention are preferably represented by the empirical formula  $(L)_p(X)_q$  where L, X, p and q are as defined above. This is intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is provided below.

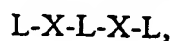
As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain or at any intermediate position thereof.

The simplest and most preferred multibinding compound is a bivalent compound which can be represented as L-X-L, where L is a ligand and is the same or different and X is the linker. A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as is X. However, a trivalent compound can also comprise three ligands attached to a central core, and thus be represented as  $(L)_3X$ , where the linker X could

include, for example, an aryl or cycloalkyl group. Tetravalent compounds can be represented in a linear array:



5 or a branched array:



|

L

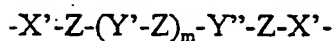
10 i.e., a branched construct analogous to the isomers of butane (*n*-butyl, *iso*-butyl, *sec*-butyl, and *t*-butyl). Alternatively, it could be represented as an aryl or cycloalkyl derivative as described above with four (4) ligands attached to the core linker.

15 The same considerations apply to higher multibinding compounds of this invention containing from 5-10 ligands. However, for multibinding agents attached to a central linker such as an aryl, cycloalkyl or heterocyclyl group, or a crown compound, there is a self-evident constraint that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of  
20 ligands.

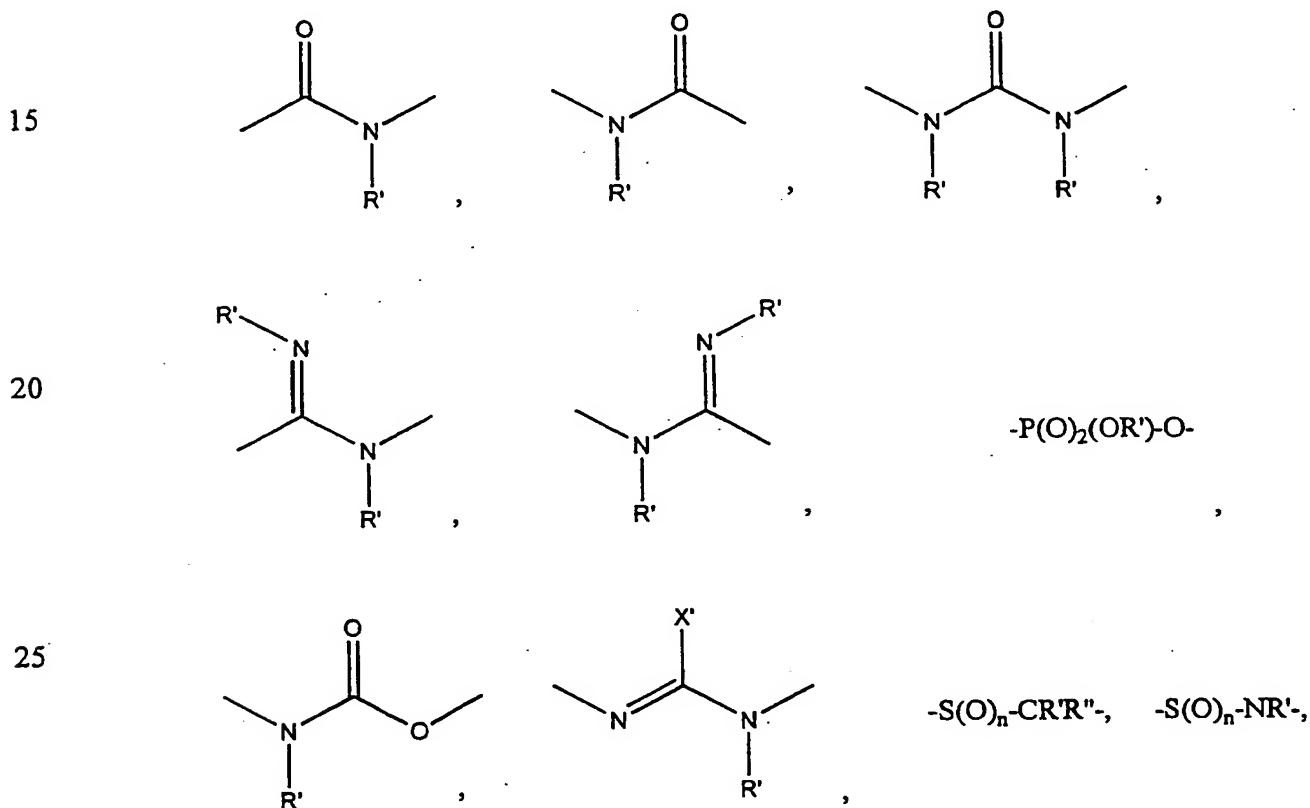
The formula  $(L)_p(X)_q$  is also intended to represent a cyclic compound of formula  $(-L-X-)_n$ , where *n* is 2-10.

25 All of the above variations are intended to be within the scope of the invention defined by the formula  $(L)_p(X)_q$ . Examples of bivalent and higher-order valency compounds of this invention are provided in Figures 4A to 4D.

With the foregoing in mind, a preferred linker may be represented by the following formula:



- 5 in which:  $m$  is an integer of from 0 to 20;  $X'$  at each separate occurrence is  $-O-$ ,  $-S-$ ,  $-S(O)-$ ,  $-S(O)_2-$ ,  $-NR-$ ,  $-N^+ R R'-$ ,  $-C(O)-$ ,  $-C(O)O-$ ,  $-C(O)NH-$ ,  $-C(S)-$ ,  $-C(S)O-$ ,  $-C(S)NH-$  or a covalent bond, where  $R$  and  $R'$  at each separate occurrence are as defined below for  $R'$  and  $R''$ ;  $Z$  is at each separate occurrence selected from alkylene, substituted alkylene, alkylalkoxy, cycloalkylene, substituted cycloalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted alkenylene, arylene, substituted arylene, heteroarylene, heterocyclene, substituted heterocyclene, crown compounds, or a covalent bond;  $Y'$  and  $Y''$  at each separate occurrence are selected from the group consisting of



-S-S- or a covalent bond; in which:  $n$  is 0, 1 or 2; and  $R'$  and  $R''$  at each separate occurrence are selected from hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl or heterocyclic.

5            Additionally, the linker moiety can be optionally substituted at any atom therein by one or more alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic group.

10            As indicated above, the simplest (and preferred) construct is a bivalent compound which can be represented as  $L-X-L$ , where  $L$  is a  $K^+$  channel ligand that is the same or different at each occurrence, and  $X$  is the linker. Accordingly, examples of the preparation of a bivalent ligand are given below as an illustration of the manner in which multibinding compounds of Formula I are obtained.

15            The reaction schemes that follow illustrate preferred linking strategies for linking phenylmethane sulfonamide (dofetilide, ibutilide, sotalol, and E-4031) and benzofuran (amiodarone, desethylamiodarone, NE-10064) classes of potassium channel modulators. These strategies are intended to apply as well to any  $K^+$  channel ligand that includes, or can be functionalized with groups compatible with the chosen linker (e.g.,  
20            azimilide and tedisamil).

                 As was previously discussed, the linker or linkers can be attached to different positions on the ligand molecule to achieve different orientations of the ligand domains and thereby facilitate multivalency. For example, the positions that are potentially available for  
25            linking a benzofuran such as amiodarone are indicated by arrows in the structure shown in Figure 5.

                 Preferred positions of attachment suggested by known SAR are illustrated in the reaction schemes of Figures 7 to 21. Examples of ligands are shown in Table 4.

Certain K<sup>+</sup> channel ligands may be chiral and exhibit stereoselectivity. The most active enantiomers are preferably used as ligands in the multibinding compounds of this invention. The chiral resolution of enantiomers is accomplished by well known procedures that result in the formation of diastereomeric derivatives or salts, followed by conventional separation by chromatographic procedures or by fractional crystallization (*see, e.g.*, Bossert, et al., *Angew. Chem. Int. Ed.*, 20:762-769 (1981) and U.S. Patent No. 5,571,827 and references cited therein). They may also be obtained by asymmetric synthesis.

The ligands are covalently attached to the linker using conventional chemical techniques. The reaction chemistries resulting in such linkage are well known in the art and involve the coupling of reactive functional groups present on the linker and ligand. In some cases, it may be necessary to protect portions of the ligand that are not involved in linking reactions. Protecting groups for this purpose are well known in the art and are indicated generally in the reaction schemes by the symbols PG and PG'.

Preferably, the reactive functional groups on the linker are selected relative to the functional groups on the ligand that are available for coupling, or can be introduced onto the ligand for this purpose. In some embodiments, the linker is coupled to ligand precursors, with the completion of ligand synthesis being carried out in a subsequent step. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as J. March, *Advanced Organic Chemistry*, 4<sup>th</sup> Ed. (Wiley- Interscience, N.Y., 1992). Examples of the chemistry for connecting ligands by a linker are shown in Figure 6, where R<sup>1</sup> and R<sup>2</sup> represent a ligand and/or the linking group. One skilled in the art will appreciate that synthetically equivalent coupling reactions can be substituted for the reactions illustrated herein.

The linker to which the ligands or ligand precursors are attached comprises a "core" molecule having two or more functional groups with reactivity that is complementary to that of the functional groups on the ligand. Figure 3 illustrates the diversity of "cores" that are



useful for varying the linker size, shape, length, orientation, rigidity, acidity/basicity, hydrophobicity/hydrophilicity, hydrogen bonding characteristics and number of ligands connected. This pictorial representation is intended only to illustrate the invention, and not to limit its scope to the structures shown. In the Figures and reaction schemes that follow, a solid circle is used to generically represent a core molecule, referred to as "Link" in the Examples. The solid circle is equivalent to a linker as defined above after reaction.

The preferred compounds of Formula I are bivalent. Accordingly, and for the purpose of simplicity, most of the figures and reaction schemes below illustrate the synthesis of bivalent  $K^+$  channel modulators. It should be noted, however, that the same techniques can be used to generate higher order multibinding compounds, i.e., the compounds of the invention where p is 3-10. (See, e.g., Figure 15 and 20.)

Reactions performed under standard amide coupling conditions are carried out in an inert polar solvent (e.g., DMF, DMA) in the presence of a hindered base (e.g., TEA, DIPEA) and standard amide coupling reagents (e.g., DPPA, PyBOP, HATU, DCC).

Several methods for preparing bivalent benzofuran (BF) compounds, as exemplified here for amiodarone and structurally analogous molecules, are illustrated in the reaction schemes for amiodarone and dronedarone shown in Figure 7. These are described in detail in Examples 1-3.

Several methods for preparing bivalent phenylmethane sulfonamide (PMS) compounds, as exemplified by dofetilide, ibutilide, sematilide and sotalol, and structurally analogous molecules are illustrated in the reaction schemes shown in Figures 8 - 11. These are described in detail in Examples 4-11.

Several methods for preparing bivalent azimilide and tedisamil compounds are illustrated in the reaction schemes shown in Figures 12 - 13. These are described in detail in Examples 12 - 14.

5           The strategies for preparing compounds of Formula I discussed above involve coupling the ligand directly to a homobifunctional core. Another strategy that can be used with all ligands, and for the preparation of both bivalent and higher order multibinding compounds, is to introduce a 'spacer' before coupling to a central core. Such a spacer can itself be selected from the same set as the possible core compounds. Examples of this  
10       linking strategy using starting materials prepared as described above, are shown in Figure 14, where the spacer is represented by a black circle. As defined herein, the linker comprises the spacer + core. These are described in detail in Examples 15-17.

15           Compounds of Formula I of higher order valency, i.e.,  $p > 2$ , can be prepared by simple extension of the above strategies. As shown in Figure 15, compounds are prepared by coupling ligands to a central core bearing multiple functional groups. The reaction conditions are the same as described above for the preparation of bivalent compounds, with appropriate adjustments made in the molar quantities of ligand and reagents. These are described in detail in Examples 18-21.

20           Figures 16 and 17 show ligands coupled to a polypeptide core with a sidechain spacer. Solid phase peptide synthesis can be used to produce a wide variety of peptidic core molecules. Techniques well-known to those skilled in the art (including combinatorial methods) are used to vary the distance between ligand attachment sites on the core molecule, the number of attachment sites available for coupling, and the chemical properties of the core  
25       molecule. Orthogonal protecting groups are used to selectively protect functional groups on the core molecule, thus allowing ancillary groups to be inserted into the linker of the multibinding compound and/or the preparation of "heterovalomers" (i.e., multibinding compounds with nonidentical ligands).

All of the synthetic strategies described above employ a step in which the ligand, attached to spacers or not, is symmetrically linked to functionally equivalent positions on a central core. Compounds of Formula I can also be synthesized using an asymmetric linear approach. This strategy is preferred when linking two or more ligands at different points of connectivity (*see, e.g.*, Figure 18) or when preparing heterovalomers (*see, e.g.*, Figure 19). These are described in detail in Examples 22-25.

#### Isolation and Purification of the Compounds

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. Characterization is preferably by NMR and mass spectroscopy.

#### Utility and Testing

The multibinding compounds of this invention can be used to modulate potassium channels in various tissues including heart, muscle, and neurons. They will typically be used for the treatment of diseases and conditions in mammals that involve or are mediated by K<sup>+</sup> channels, such as hypertension, cardiac arrhythmias, cerebral ischemia, congestive heart failure, and the like.

The multibinding compounds of this invention are tested in well-known and reliable assays and their activities are compared with those of the corresponding unlinked (i.e., monovalent) ligands.

#### Binding affinity to potassium channels

The binding affinity is determined by a radioligand competitive inhibition assay.<sup>23</sup> The ability of the present compounds to compete with [<sup>3</sup>H]dofetilide or a similar radioactive ligand in binding to high- and low-affinity binding sites of guinea pig ventricular myocytes is

measured *in vitro*. The binding affinity, calculated from competition curves, is compared with that of the monovalent ligand and/or monovalent linker-ligand conjugate.

#### Antiarrhythmic effect

5           Antiarrhythmic effect of compounds of this invention may be determined *in vivo* in dogs with induced myocardial infarction and reproducibly inducible ventricular tachycardia or ventricular fibrillation.<sup>1,22</sup> Suppression of inducible arrhythmias is measured.

10           The antifibrillatory and antiarrhythmic effects of the compounds of this invention may be determined *in vivo* using a canine model of sudden death.<sup>7</sup> Reduction of the incidence of programmed electrical stimulation (PES) induced ventricular tachycardia and protection against ischemia-induced ventricular fibrillation are measured.

15           The antiarrhythmic effect of the compounds of this invention may be determined *in vivo* using the mouse chloroform model.<sup>8</sup> The percentage of animals showing normal sinus rhythm is measured.

20           The antiarrhythmic effect of the compounds of this invention may be determined *in vivo* using the rat coronary ligation model.<sup>8</sup> Ventricular extrasystoles occurring during the 30 minutes following the procedure are counted.

25           The antiarrhythmic effect of the compounds of this invention may be determined *in vitro* or *in vivo* using rat coronary artery ligation/reperfusion models.<sup>8</sup> In the *in vitro* model, excised rat hearts are retrogradely perfused with a solution of the compound to be tested, then the coronary artery is ligated, followed by reperfusion. In the *in vivo* evaluation, the compound is administered i.p., then the coronary artery is ligated, followed by reperfusion. In both models the incidence and time to onset during reperfusion of ventricular extrasystole, tachyarrhythmia and fibrillation are measured.

The antiarrhythmic effect of the compounds of this invention may be determined *in vivo* using an anesthetized rat model of ventricular arrhythmias.<sup>9</sup> The time to onset of ventricular extrasystoles is measured.

5        The antiarrhythmic effect of the compounds of this invention may be determined *in vivo* using a canine myocardial infarction model where compound is administered 24 hours after ligation of the left anterior descending coronary artery.<sup>11</sup> Right ventricular effective refractory period, monophasic action potential duration and reduction of PES induced ventricular tachycardia and ventricular fibrillation are measured.

10

The ability of the compounds of this invention to prolong the action potential (achieve a slower onset of active state block) and recover faster from block may be determined *in vitro* using rabbit ventricular myocytes.<sup>13,30</sup> Development of block during a long depolarizing clamp and recovery from block are measured.

15

The ability of the compounds of this invention to suppress repolarization arrhythmias may be determined *in vitro* using canine epicardium midmyocardium and endocardium and canine cardiac Purkinje fibers and *in vivo* using anesthetized rabbits.<sup>26,58,62</sup>

20

The ability of the compounds of this invention to suppress arrhythmias may be determined *in vivo* using the feline coronary occlusion and left stellate ganglion stimulation model, the conscious canine model of transient ischemia during exercise in the presence of a healed MI and the conscious canine model of complete occlusion after recent MI.<sup>52,59</sup>

25

The ability of the compounds of this invention to prolong action potential duration may be determined *in vivo* and *in vitro* using guinea pig hearts,<sup>57</sup> and *in vitro* using calf cardiac Purkinje fibers.<sup>63</sup>

The ability of the compounds of this invention to prevent atrial fibrillation (AF) may be determined using a canine model of sustained vagotonic AF.<sup>68</sup> Prevention of AF induction is measured. Reverse use dependence may also be determined.

5     Effect on tachycardia

The effect of compounds of this invention on tachycardia may be determined *in vitro* using rabbit right atrial preparations.<sup>2</sup> Micro-electrode techniques are used to measure the ability to prolong the refractory period and thus prevent initiation of tachycardia.

10     The effect of the compounds of this invention on tachyarrhythmias may be determined *in vitro* using guinea pig right ventricular papillary muscle.<sup>27</sup> The action potential duration at different extracellular potassium concentrations is measured.

Effect on potassium currents

15     The effect of compounds of this invention on the repolarization currents  $I_K$  and  $I_{To}$  may be determined *in vitro* using whole cell recordings in cat ventricular myocytes and papillary muscles from the hearts of oophorectomized rabbits.<sup>4,31</sup>

20     The effect of compounds of this invention on various specific potassium current may be determined *in vitro* using guinea pig ventricular myocytes and sinoatrial node cells, human atrial myocytes, canine ventricular muscle and Purkinje fibers, guinea pig papillary muscle, single voltage clamped guinea pig ventricular myocytes and human ventricular endomyocardium.<sup>6,12,17,23,29,32,33,37,51,66,69,71</sup>

25     The ability of compounds of this invention to inhibit potassium currents in a non-cardiac preparation may be determined using rat taste receptor cells.<sup>60</sup>

Selectivity and/or Specificity:

The ability of compounds of this invention to modulate the KATP channel may be determined using a  $^{86}\text{Rb}$  efflux assay.<sup>15,64</sup> Thus, this is a potency assay.

5        The selectivity and/or specificity of the compounds of this invention may be determined using CHO cell lines expressing specific recombinant potassium channel subtypes.<sup>34</sup>

10       The selectivity of compounds of this invention for various potassium channel currents may be determined *in vitro* using cloned K channels expressed in cells or ventricular myocytes.<sup>12,34</sup>

15       The selectivity of compounds of this invention for various receptors may be determined *in vitro* using rat synaptosomal membrane. (Pong, et al. "Binding profile of NE-10064, a novel Class III anti-arrhythmic agent to rat brain receptors", *Faseb J.*, 7:A474 (1993)).

Antivasoconstrictor activity

20       Antivasoconstrictor activity is determined as described in Brittain, et al., *Physiologist*, 28:325 (1985) as the concentration of a compound required to produce 50% vasorelaxation in KCl-contracted rabbit thoracic aorta strips in the presence of calcium. Alternatively, the concentration of a compound required to inhibit coronary vasoconstriction induced by a thromboxane mimetic (U-46619, i.e., 9,11-methanoepoxy-PGH<sub>2</sub>) in guinea pig Langendorff heart preparation is measured as described in Eltze, et al., *Chirality*, 2:233-240 (1990).

25

Antihypertensive activity

Antihypertensive activity is determined in male spontaneously hypertensive rats by measurement of mean arterial blood pressure (Rovnyak, et al., *J. Med. Chem.*, 35:3254-3263 (1992)).

### Tissue selectivity

Selectivity for vascular smooth muscle as compared with cardiac muscle can be assessed by comparing the concentration of a multibinding compound that produces a 50% increase in coronary blood flow in an isolated guinea-pig heart with that required to inhibit myocardial contractility. See, e.g., Osterrieder, W. and Holck, M., *J. Cardiovasc. Pharm.*, 13:754-9 (1989); and Cremers, et al., *J. Cardiovasc. Pharm.*, 29:692-696 (1997).

### Combinatorial Libraries

The methods described above lend themselves to combinatorial approaches for identifying multimeric compounds which possess multibinding properties for potassium channels.

Specifically, factors such as the proper juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding sites on a target or targets is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for a particular target. These parameters include: (1) the identity of ligand(s), (2) the orientation of ligands, (3) the valency of the construct, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:



### Selection of ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed against a particular biological target or targets. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s). Thus, ligands may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short *in vivo* half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

### Orientation: selection of ligand attachment points and linking chemistry

Several points are chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting

the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a protease inhibitor bound to its target allows one to identify one or more sites where linker attachment will not preclude the enzyme:inhibitor interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a receptor antagonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the formal antagonist binding

site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in monomeric form.

5           The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands that bind to common or different targets. For  
10           example, a 5HT<sub>4</sub> receptor antagonist and a bladder-selective muscarinic M<sub>3</sub> antagonist may be joined to a linker through attachment points which do not abrogate the binding affinity of the monomeric ligands for their respective receptor sites. The dimeric compound may achieve enhanced affinity for both receptors due to favorable interactions between the 5HT<sub>4</sub> ligand and elements of the M<sub>3</sub> receptor proximal to the formal M<sub>3</sub> antagonist binding site  
15           and between the M<sub>3</sub> ligand and elements of the 5HT<sub>4</sub> receptor proximal to the formal 5HT<sub>4</sub> antagonist binding site. Thus, the dimeric compound may be more potent and selective antagonist of overactive bladder and a superior therapy for urinary urge incontinence.

20           Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically innocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are  
25           but a few examples of preferred linkages.

Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into  
5 consideration the following factors:

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than  
10 sufficient to confer biological advantage. Furthermore, divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the spanning of a  
15 range of inter-ligand distances that encompass the distance preferable for a given divalent interaction. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets, typically enzymes and soluble receptor targets. In other instances where high-resolution structural information is not available (such as 7TM G-protein coupled receptors), one can make use of simple models to estimate  
20 the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are 2-20 Å, with more preferred linker distances of 3-12 Å. In situations where two binding sites reside on separate (e.g., protein) target sites, preferred linker distances are 20-100 Å, with  
25 more preferred distances of 30-70 Å.

Linker geometry and rigidity. The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in which the

ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two ligands to the ortho, meta, and para positions of a benzene ring, or in *cis*- or *trans*-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around a cyclohexane core or in *cis*- or *trans*-arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

Linker physical properties. The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarization, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

### Combinatorial synthesis

Having chosen a set of  $n$  ligands ( $n$  being determined by the sum of the number of different attachment points for each ligand chosen) and  $m$  linkers by the process outlined above, a library of  $(n!)m$  candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalities on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or linker is attached to a solid support. Alternatively and preferably, the combinatorial library is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

### Analysis of array by biochemical, analytical, pharmacological, and computational methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess

multibinding properties. Physical constants such as solubility under various solvent conditions and logD/clogD values can be determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition analysis for many enzyme targets. *In vitro* efficacy, such as for receptor agonists and antagonists, ion channel blockers, and antimicrobial activity, can also be determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data can be determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

The members of the library which exhibit multibinding properties, as defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both *in vitro* and *in vivo*).

Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libraries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No.

2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative binding affinities of candidate multibinding compounds to receptors.

5           The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

Follow-up synthesis and analysis of additional array(s)

10           Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries can then be generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure in an effort to further optimize target

15           affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

20

          To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates, diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case,

25           the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:



## COMPLEMENTARY BINDING CHEMISTRIES

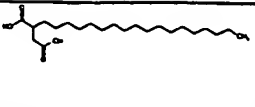
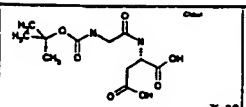
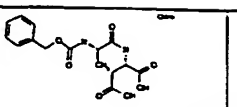
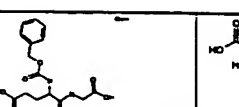
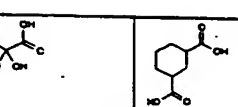

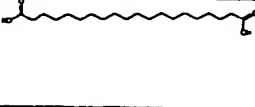
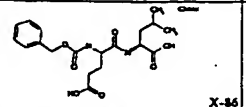
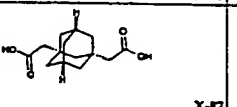
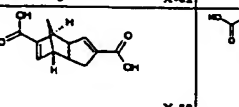
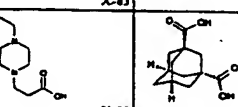

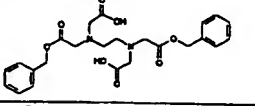
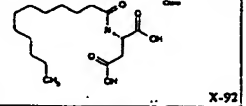
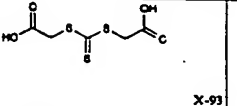
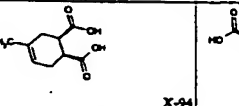
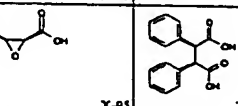

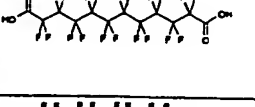
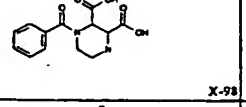
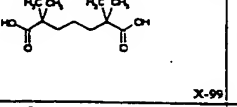
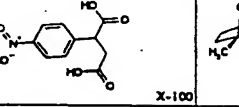
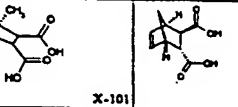

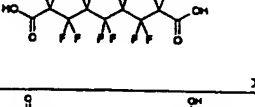
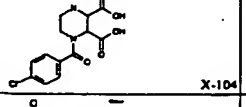
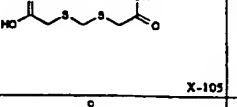
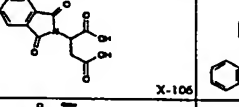
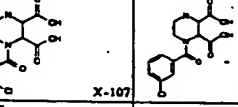

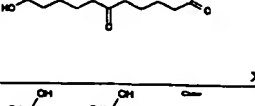
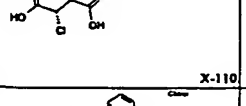
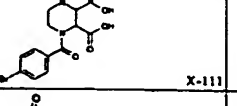
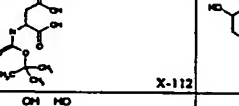
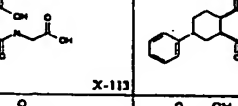

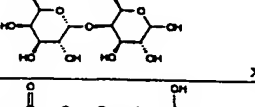
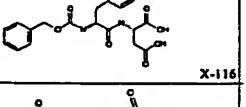
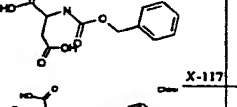
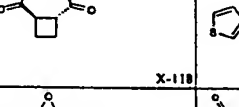
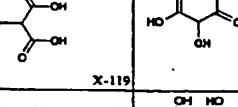
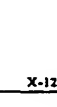
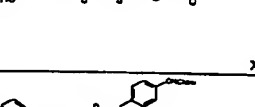
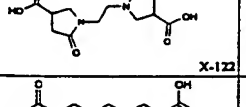
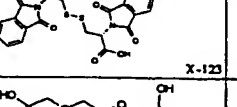
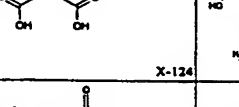
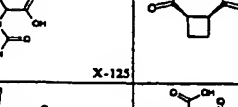
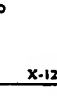
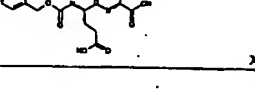
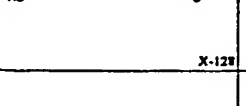
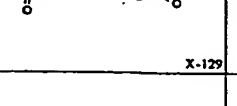
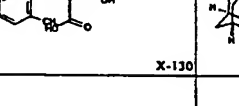
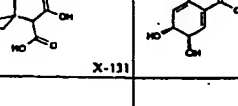
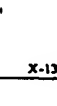
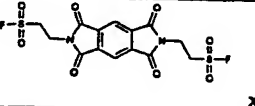
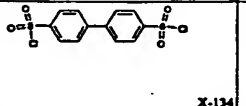
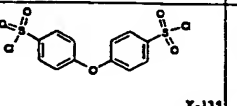
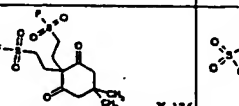
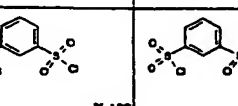
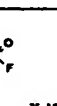
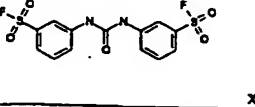
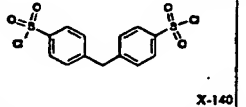
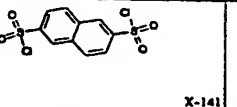
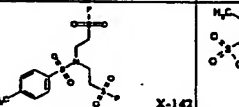
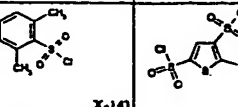
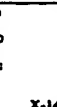
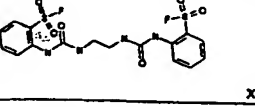
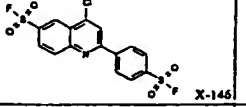
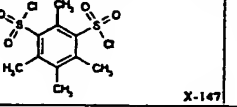
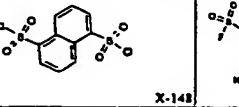
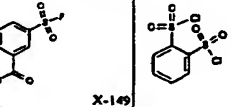

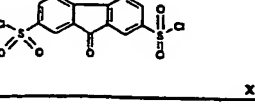
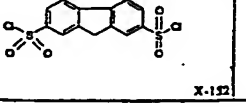
	<u>First Reactive Group</u>	<u>Second Reactive Group</u>	<u>Linkage</u>
5	hydroxyl	isocyanate	urethane
	amine	epoxide	$\beta$ -hydroxyamine
	sulfonyl halide	amine	sulfonamide
	carboxyl acid	amine	amide
	hydroxyl	alkyl/aryl halide	ether
	aldehyde	amine/ $\text{NaCNBH}_4$	amine
10	ketone	amine/ $\text{NaCNBH}_4$	amine
	amine	isocyanate	carbamate

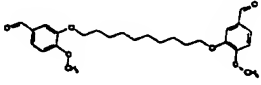
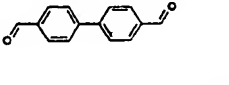
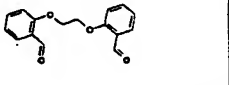
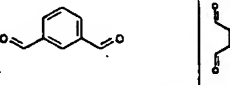
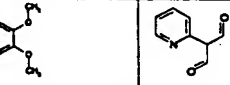

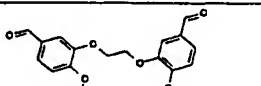
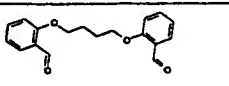
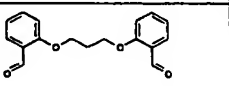
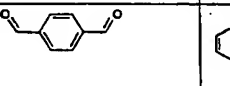
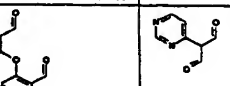

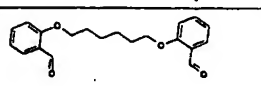
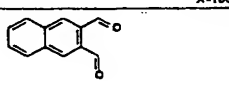
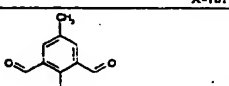
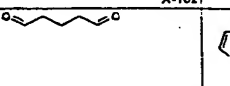
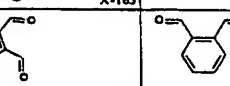

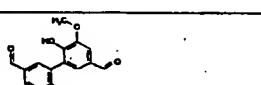
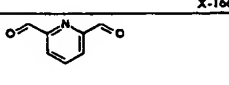
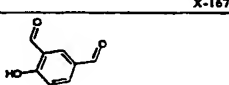
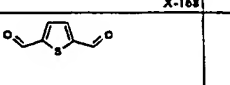
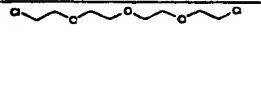
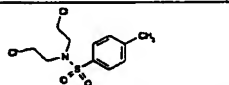
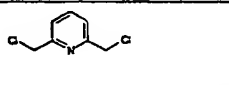
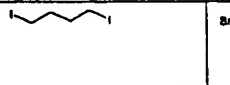


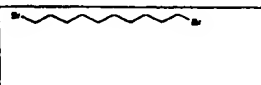
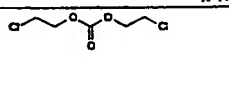
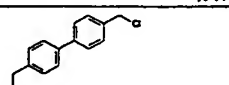
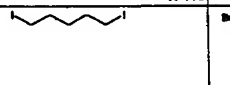
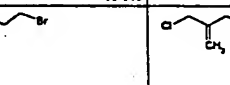

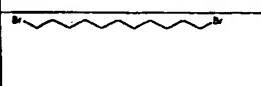
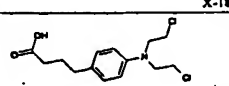
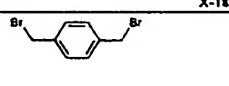
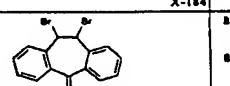
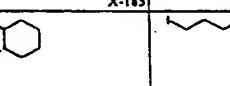

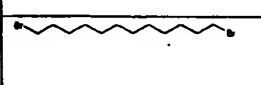
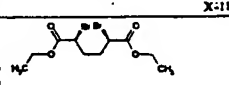
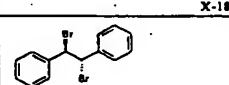
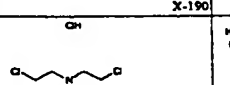
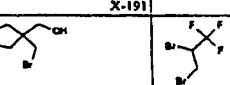

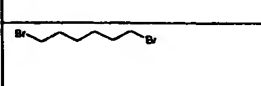
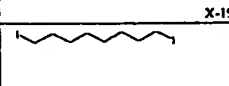
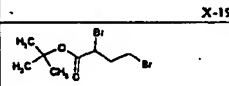
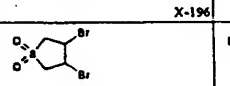
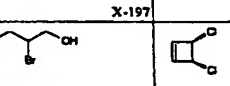

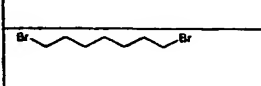
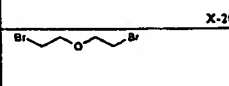
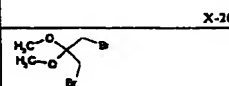
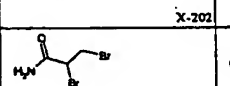
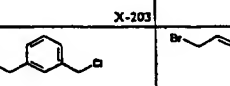

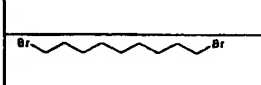
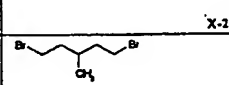
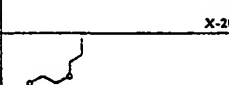
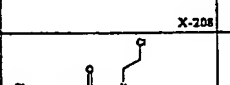
Exemplary linkers include the following linkers identified as X-1 through X-418 as set forth below:

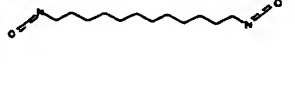


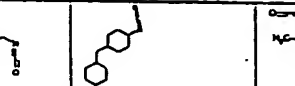

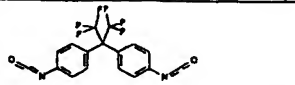
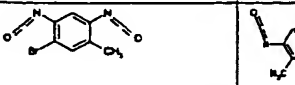
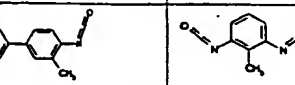
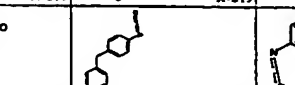
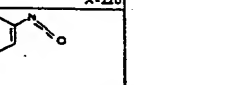

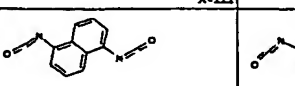
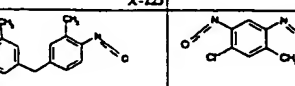
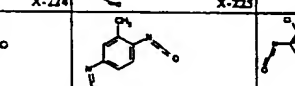
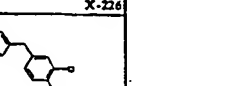
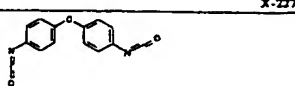
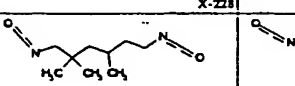
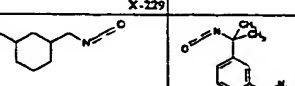
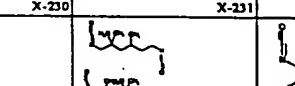
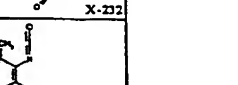

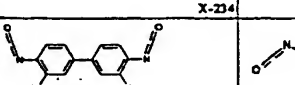
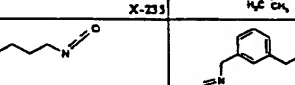
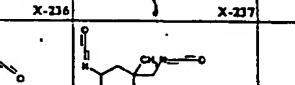
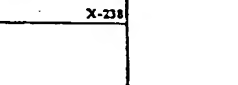
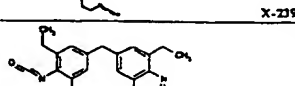
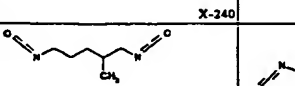
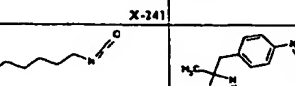
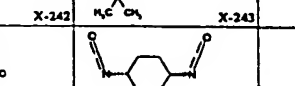
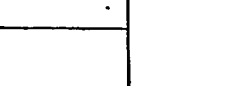
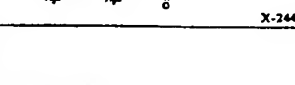
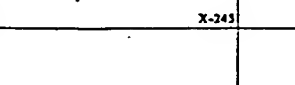
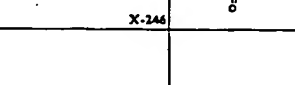
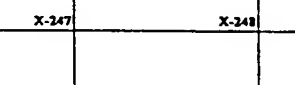

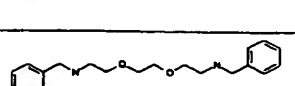
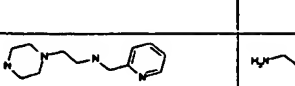
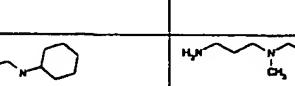
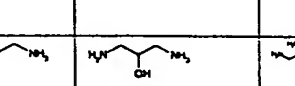

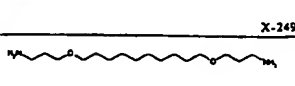

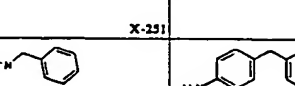
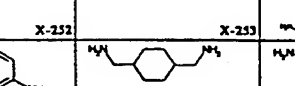
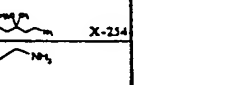
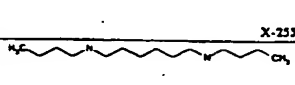
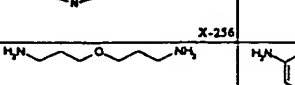
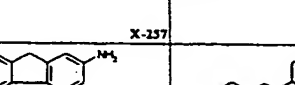
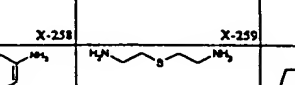
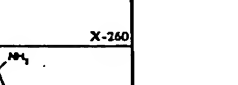
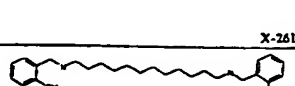
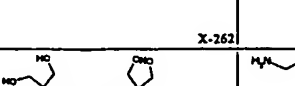
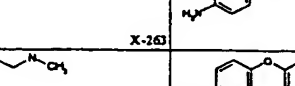
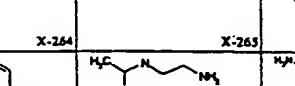
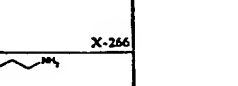
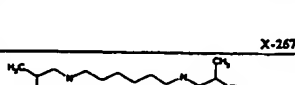
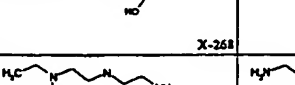
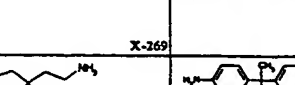

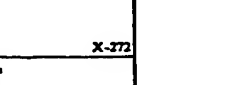
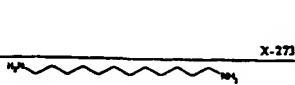
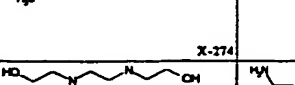
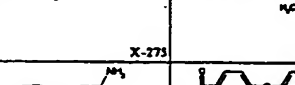

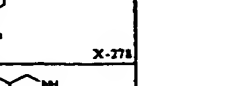
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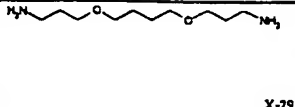
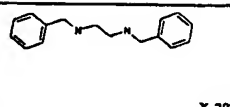
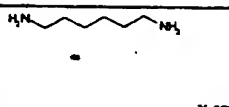
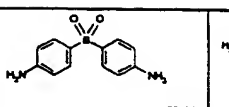
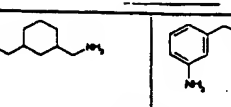
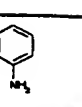
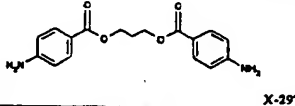
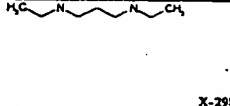
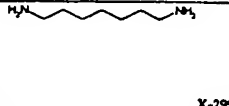
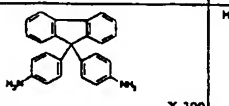
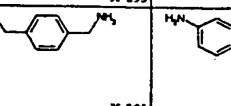
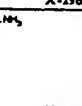
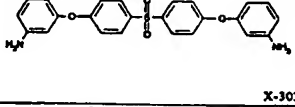
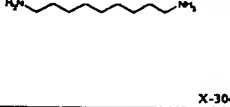
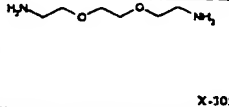
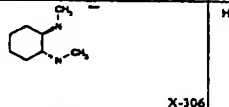
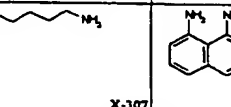

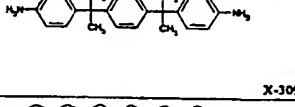
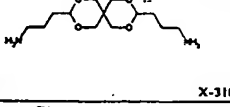
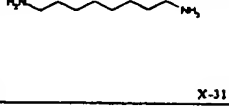
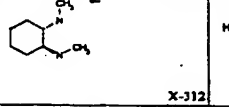
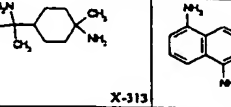

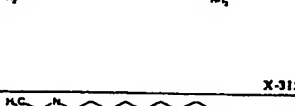
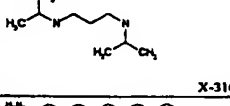
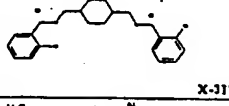
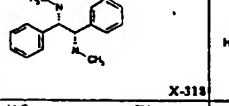
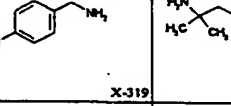
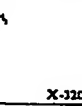


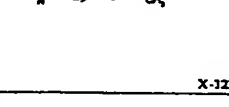
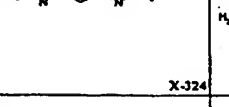
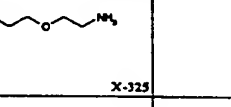
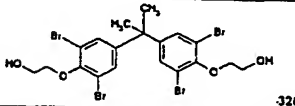
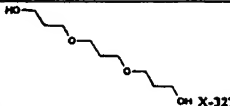
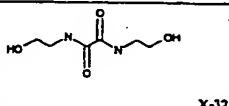
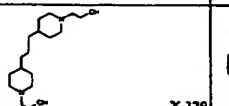
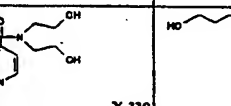
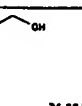
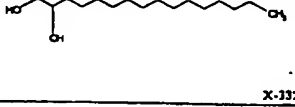
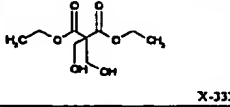
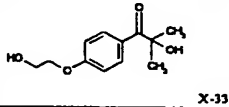
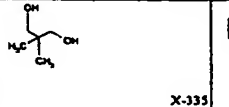
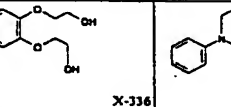
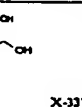
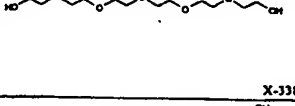
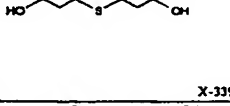
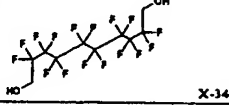
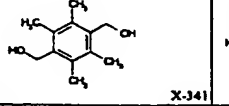
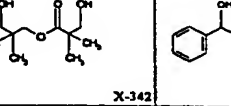

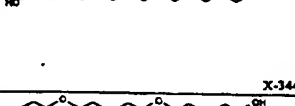
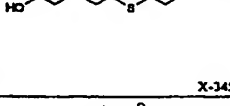
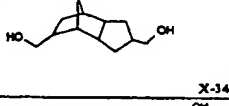
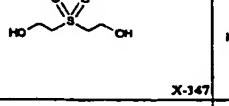
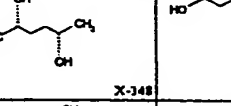
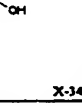
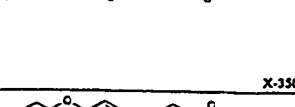
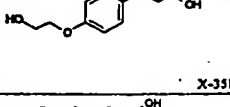
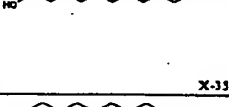
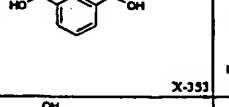
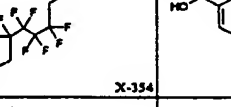
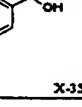
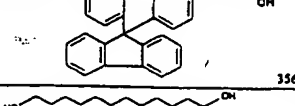
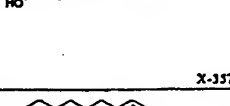
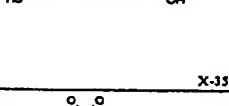
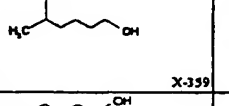
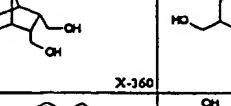
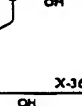

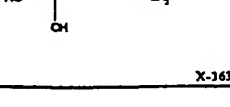
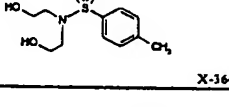
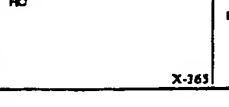
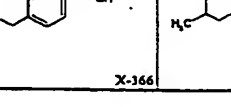
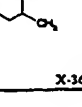
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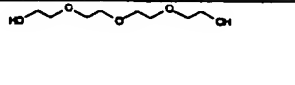
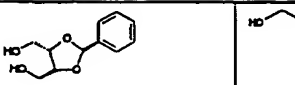
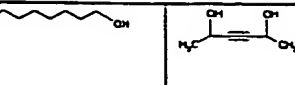
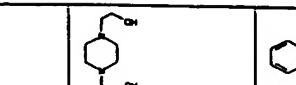
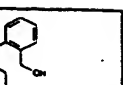

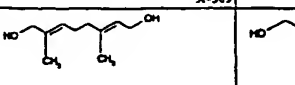
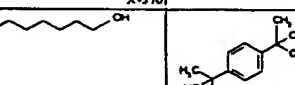
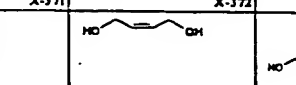
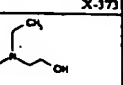
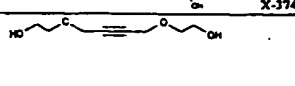
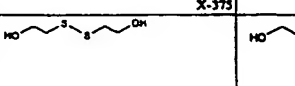
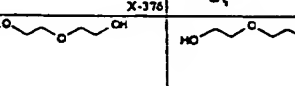
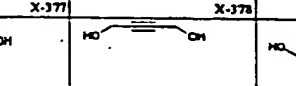
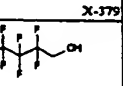
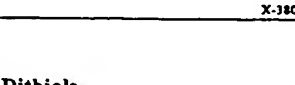
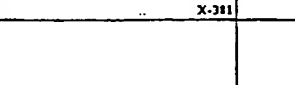
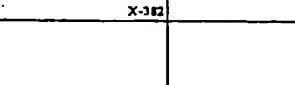
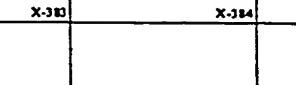
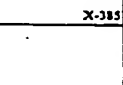
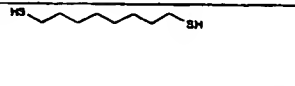
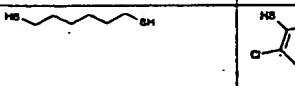
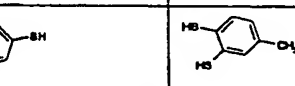
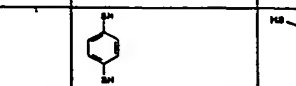
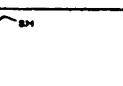
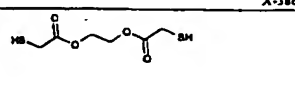
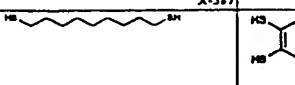
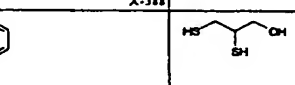
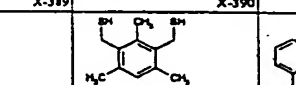
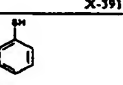
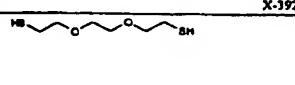
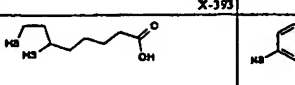
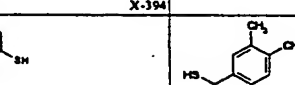

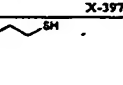
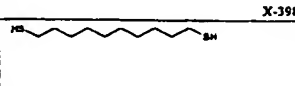
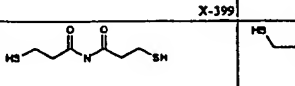
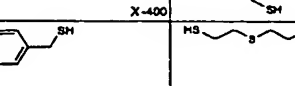
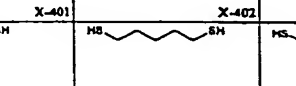
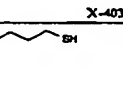
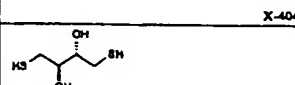
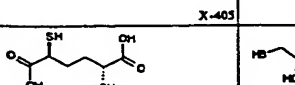
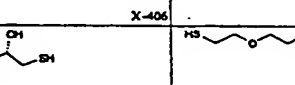
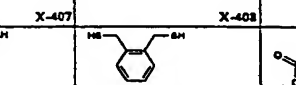
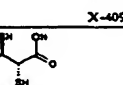
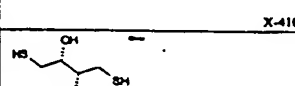
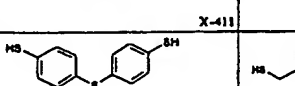
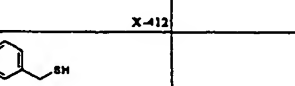
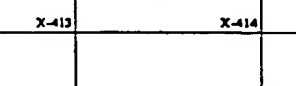
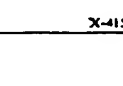
Diacids					
X-1	X-2	X-3	X-4	X-5	X-6
X-7	X-8	X-9	X-10	X-11	X-12
X-13	X-14	X-15	X-16	X-17	X-18
X-19	X-20	X-21	X-22	X-23	X-24
X-25	X-26	X-27	X-28	X-29	X-30
X-31	X-32	X-33	X-34	X-35	X-36
X-37	X-38	X-39	X-40	X-41	X-42
X-43	X-44	X-45	X-46	X-47	X-48
X-49	X-50	X-51	X-52	X-53	X-54
X-55	X-56	X-57	X-58	X-59	X-60
X-61	X-62	X-63	X-64	X-65	X-66
X-67	X-68	X-69	X-70	X-71	X-72
X-73	X-74	X-75	X-76	X-77	X-78

					
X-79	X-80	X-81	X-82	X-83	X-84
					
X-85	X-86	X-87	X-88	X-89	X-90
					
X-91	X-92	X-93	X-94	X-95	X-96
					
X-97	X-98	X-99	X-100	X-101	X-102
					
X-103	X-104	X-105	X-106	X-107	X-108
					
X-109	X-110	X-111	X-112	X-113	X-114
					
X-115	X-116	X-117	X-118	X-119	X-120
					
X-121	X-122	X-123	X-124	X-125	X-126
					
X-127	X-128	X-129	X-130	X-131	X-132
<b>Disulfonyl Halides</b>					
					
X-133	X-134	X-135	X-136	X-137	X-138
					
X-139	X-140	X-141	X-142	X-143	X-144
					
X-145	X-146	X-147	X-148	X-149	X-150
					
X-151	X-152				

<b>Dialdehydes</b>					
					
X-153	X-154	X-155	X-156	X-157	X-158
					
X-159	X-160	X-161	X-162	X-163	X-164
					
X-165	X-166	X-167	X-168	X-169	X-170
					
X-171	X-172	X-173	X-174		
<b>Dihalides</b>					
					
X-175	X-176	X-177	X-178	X-179	X-180
					
X-181	X-182	X-183	X-184	X-185	X-186
					
X-187	X-188	X-189	X-190	X-191	X-192
					
X-193	X-194	X-195	X-196	X-197	X-198
					
X-199	X-200	X-201	X-202	X-203	X-204
					
X-205	X-206	X-207	X-208	X-209	X-210
					
X-211	X-212	X-213	X-214		
<b>Diisocyanates</b>					

					
X-213	X-216	X-217	X-218	X-219	X-220
					
X-221	X-222	X-223	X-224	X-225	X-226
					
X-227	X-228	X-229	X-230	X-231	X-232
					
X-233	X-234	X-235	X-236	X-237	X-238
					
X-239	X-240	X-241	X-242	X-243	
					
X-244	X-245	X-246	X-247	X-248	
<b>Diamines</b>					
					
X-249	X-250	X-251	X-252	X-253	X-254
					
X-255	X-256	X-257	X-258	X-259	X-260
					
X-261	X-262	X-263	X-264	X-265	X-266
					
X-267	X-268	X-269	X-270	X-271	X-272
					
X-273	X-274	X-275	X-276	X-277	X-278
					
X-279	X-280	X-281	X-282	X-283	X-284
					
X-285	X-286	X-287	X-288	X-289	X-290

					
X-291	X-292	X-293	X-294	X-295	X-296
					
X-297	X-298	X-299	X-300	X-301	X-302
					
X-303	X-304	X-305	X-306	X-307	X-308
					
X-309	X-310	X-311	X-312	X-313	X-314
					
X-315	X-316	X-317	X-318	X-319	X-320
					
X-321	X-322	X-323	X-324	X-325	
<b>Diols</b>					
					
X-326	X-327	X-328	X-329	X-330	X-331
					
X-332	X-333	X-334	X-335	X-336	X-337
					
X-338	X-339	X-340	X-341	X-342	X-343
					
X-344	X-345	X-346	X-347	X-348	X-349
					
X-350	X-351	X-352	X-353	X-354	X-355
					
X-356	X-357	X-358	X-359	X-360	X-361
					
X-362	X-363	X-364	X-365	X-366	X-367

				
X-368	X-369	X-370	X-371	X-372
				
X-373	X-374	X-375	X-376	X-377
				
X-378	X-379	X-380	X-381	X-382
				
X-383	X-384	X-385	X-386	X-387
<b>Dithiols</b>				
				
X-388	X-389	X-390	X-391	X-392
				
X-393	X-394	X-395	X-396	X-397
				
X-398	X-399	X-400	X-401	X-402
				
X-403	X-404	X-405	X-406	X-407
				
X-408	X-409	X-410	X-411	X-412
				
X-413	X-414	X-415	X-416	X-417

Representative ligands for use in this invention include, by way of example, L-1 through L-4. L-1 ligands are benzofuran compounds (e.g., 7A-1, 7B-1 or 7C-1 of Examples 1-3). Phenylmethane sulfonamide structures are designated L-2 ligands (e.g., 8A-1, 8B-1, 8C-1, 9A-1, 9B-1, 10A-1, 10B-1, or 11-1 of Examples 4-11). L-3 ligands are azimilide compounds (e.g., 12-1, 12-3 of Examples 12-13). L-4 ligands are tedisamil compounds (e.g., 13-1 of Example 14).

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 through L-4 above and the second ligand and linker is selected from the following:

	L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
	L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
	L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
15	L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
	L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
	L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
	L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
	L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
20	L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
	L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
	L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
	L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
	L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
25	L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
	L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-
	L-1/X-91-	L-1/X-92-	L-1/X-93-	L-1/X-94-	L-1/X-95-	L-1/X-96-
	L-1/X-97-	L-1/X-98-	L-1/X-99-	L-1/X-100-	L-1/X-101-	L-1/X-102-



	L-1/X-103-	L-1/X-104-	L-1/X-105-	L-1/X-106-	L-1/X-107-	L-1/X-108-
	L-1/X-109-	L-1/X-110-	L-1/X-111-	L-1/X-112-	L-1/X-113-	L-1/X-114-
	L-1/X-115-	L-1/X-116-	L-1/X-117-	L-1/X-118-	L-1/X-119-	L-1/X-120-
	L-1/X-121-	L-1/X-122-	L-1/X-123-	L-1/X-124-	L-1/X-125-	L-1/X-126-
5	L-1/X-127-	L-1/X-128-	L-1/X-129-	L-1/X-130-	L-1/X-131-	L-1/X-132-
	L-1/X-133-	L-1/X-134-	L-1/X-135-	L-1/X-136-	L-1/X-137-	L-1/X-138-
	L-1/X-139-	L-1/X-140-	L-1/X-141-	L-1/X-142-	L-1/X-143-	L-1/X-144-
	L-1/X-145-	L-1/X-146-	L-1/X-147-	L-1/X-148-	L-1/X-149-	L-1/X-150-
	L-1/X-151-	L-1/X-152-	L-1/X-153-	L-1/X-154-	L-1/X-155-	L-1/X-156-
10	L-1/X-157-	L-1/X-158-	L-1/X-159-	L-1/X-160-	L-1/X-161-	L-1/X-162-
	L-1/X-163-	L-1/X-164-	L-1/X-165-	L-1/X-166-	L-1/X-167-	L-1/X-168-
	L-1/X-169-	L-1/X-170-	L-1/X-171-	L-1/X-172-	L-1/X-173-	L-1/X-174-
	L-1/X-175-	L-1/X-176-	L-1/X-177-	L-1/X-178-	L-1/X-179-	L-1/X-180-
	L-1/X-181-	L-1/X-182-	L-1/X-183-	L-1/X-184-	L-1/X-185-	L-1/X-186-
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	L-1/X-211-	L-1/X-212-	L-1/X-213-	L-1/X-214-	L-1/X-215-	L-1/X-216-
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	L-1/X-253-	L-1/X-254-	L-1/X-255-	L-1/X-256-	L-1/X-257-	L-1/X-258-
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	L-1/X-331-	L-1/X-332-	L-1/X-333-	L-1/X-334-	L-1/X-335-	L-1/X-336-
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	L-1/X-349-	L-1/X-350-	L-1/X-351-	L-1/X-352-	L-1/X-353-	L-1/X-354-
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	L-2/X-1-	L-2/X-2-	L-2/X-3-	L-2/X-4-	L-2/X-5-	L-2/X-6-
	L-2/X-7-	L-2/X-8-	L-2/X-9-	L-2/X-10-	L-2/X-11-	L-2/X-12-

	L-2/X-13-	L-2/X-14-	L-2/X-15-	L-2/X-16-	L-2/X-17-	L-2/X-18-
	L-2/X-19-	L-2/X-20-	L-2/X-21-	L-2/X-22-	L-2/X-23-	L-2/X-24-
	L-2/X-25-	L-2/X-26-	L-2/X-27-	L-2/X-28-	L-2/X-29-	L-2/X-30-
	L-2/X-31-	L-2/X-32-	L-2/X-33-	L-2/X-34-	L-2/X-35-	L-2/X-36-
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	L-2/X-55-	L-2/X-56-	L-2/X-57-	L-2/X-58-	L-2/X-59-	L-2/X-60-
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	L-2/X-79-	L-2/X-80-	L-2/X-81-	L-2/X-82-	L-2/X-83-	L-2/X-84-
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	L-2/X-169	L-2/X-170	L-2/X-171	L-2/X-172	L-2/X-173-	L-2/X-174-
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	L-3/X-187-	L-3/X-188-	L-3/X-189-	L-3/X-190-	L-3/X-191-	L-3/X-192-
	L-3/X-193-	L-3/X-194-	L-3/X-195-	L-3/X-196-	L-3/X-197-	L-3/X-198-
	L-3/X-199-	L-3/X-200-	L-3/X-201-	L-3/X-202-	L-3/X-203-	L-3/X-204-
20	L-3/X-205-	L-3/X-206-	L-3/X-207-	L-3/X-208-	L-3/X-209-	L-3/X-210-
	L-3/X-211-	L-3/X-212-	L-3/X-213-	L-3/X-214-	L-3/X-215-	L-3/X-216-
	L-3/X-217-	L-3/X-218-	L-3/X-219-	L-3/X-220-	L-3/X-221-	L-3/X-222-
	L-3/X-223-	L-3/X-224-	L-3/X-225-	L-3/X-226-	L-3/X-227-	L-3/X-228-
	L-3/X-229-	L-3/X-230-	L-3/X-231-	L-3/X-232-	L-3/X-233-	L-3/X-234-
25	L-3/X-235-	L-3/X-236-	L-3/X-237-	L-3/X-238-	L-3/X-239-	L-3/X-240-
	L-3/X-241-	L-3/X-242-	L-3/X-243-	L-3/X-244-	L-3/X-245-	L-3/X-246-
	L-3/X-247-	L-3/X-248-	L-3/X-249-	L-3/X-250-	L-3/X-251-	L-3/X-252-
	L-3/X-253-	L-3/X-254-	L-3/X-255-	L-3/X-256-	L-3/X-257-	L-3/X-258-

	L-3/X-259-	L-3/X-260-	L-3/X-261-	L-3/X-262-	L-3/X-263-	L-3/X-264-
	L-3/X-265-	L-3/X-266-	L-3/X-267-	L-3/X-268-	L-3/X-269-	L-3/X-270-
	L-3/X-271-	L-3/X-272-	L-3/X-273-	L-3/X-274-	L-3/X-275-	L-3/X-276-
	L-3/X-277-	L-3/X-278-	L-3/X-279-	L-3/X-280-	L-3/X-281-	L-3/X-282-
5	L-3/X-283-	L-3/X-284-	L-3/X-285-	L-3/X-286-	L-3/X-287-	L-3/X-288-
	L-3/X-289-	L-3/X-290-	L-3/X-291-	L-3/X-292-	L-3/X-293-	L-3/X-294-
	L-3/X-295-	L-3/X-296-	L-3/X-297-	L-3/X-298-	L-3/X-299-	L-3/X-300-
	L-3/X-301-	L-3/X-302-	L-3/X-303-	L-3/X-304-	L-3/X-305-	L-3/X-306-
	L-3/X-307-	L-3/X-308-	L-3/X-309-	L-3/X-310-	L-3/X-311-	L-3/X-312-
10	L-3/X-313-	L-3/X-314-	L-3/X-315-	L-3/X-316-	L-3/X-317-	L-3/X-318-
	L-3/X-319-	L-3/X-320-	L-3/X-321-	L-3/X-322-	L-3/X-323-	L-3/X-324-
	L-3/X-325-	L-3/X-326-	L-3/X-327-	L-3/X-328-	L-3/X-329-	L-3/X-330-
	L-3/X-331-	L-3/X-332-	L-3/X-333-	L-3/X-334-	L-3/X-335-	L-3/X-336-
	L-3/X-337-	L-3/X-338-	L-3/X-339-	L-3/X-340-	L-3/X-341-	L-3/X-342-
15	L-3/X-343-	L-3/X-344-	L-3/X-345-	L-3/X-346-	L-3/X-347-	L-3/X-348-
	L-3/X-349-	L-3/X-350-	L-3/X-351-	L-3/X-352-	L-3/X-353-	L-3/X-354-
	L-3/X-355-	L-3/X-356-	L-3/X-357-	L-3/X-358-	L-3/X-359-	L-3/X-360-
	L-3/X-361-	L-3/X-362-	L-3/X-363-	L-3/X-364-	L-3/X-365-	L-3/X-366-
	L-3/X-367-	L-3/X-368-	L-3/X-369-	L-3/X-370-	L-3/X-371-	L-3/X-372-
20	L-3/X-373-	L-3/X-374-	L-3/X-375-	L-3/X-376-	L-3/X-377-	L-3/X-378-
	L-3/X-379-	L-3/X-380-	L-3/X-381-	L-3/X-382-	L-3/X-383-	L-3/X-384-
	L-3/X-385-	L-3/X-386-	L-3/X-387-	L-3/X-388-	L-3/X-389-	L-3/X-390-
	L-3/X-391-	L-3/X-392-	L-3/X-393-	L-3/X-394-	L-3/X-395-	L-3/X-396-
	L-3/X-397-	L-3/X-398-	L-3/X-399-	L-3/X-400-	L-3/X-401-	L-3/X-402-
25	L-3/X-403-	L-3/X-404-	L-3/X-405-	L-3/X-406-	L-3/X-407-	L-3/X-408-
	L-3/X-409-	L-3/X-410-	L-3/X-411-	L-3/X-412-	L-3/X-413-	L-3/X-414-
	L-3/X-415-	L-3/X-416-	L-3/X-417-	L-3/X-418-		

	L-4/X-1-	L-4/X-2-	L-4/X-3-	L-4/X-4-	L-4/X-5-	L-4/X-6-
	L-4/X-7-	L-4/X-8-	L-4/X-9-	L-4/X-10-	L-4/X-11-	L-4/X-12-
	L-4/X-13-	L-4/X-14-	L-4/X-15-	L-4/X-16-	L-4/X-17-	L-4/X-18-
	L-4/X-19-	L-4/X-20-	L-4/X-21-	L-4/X-22-	L-4/X-23-	L-4/X-24-
5	L-4/X-25-	L-4/X-26-	L-4/X-27-	L-4/X-28-	L-4/X-29-	L-4/X-30-
	L-4/X-31-	L-4/X-32-	L-4/X-33-	L-4/X-34-	L-4/X-35-	L-4/X-36-
	L-4/X-37-	L-4/X-38-	L-4/X-39-	L-4/X-40-	L-4/X-41-	L-4/X-42-
	L-4/X-43-	L-4/X-44-	L-4/X-45-	L-4/X-46-	L-4/X-47-	L-4/X-48-
	L-4/X-49-	L-4/X-50-	L-4/X-51-	L-4/X-52-	L-4/X-53-	L-4/X-54-
10	L-4/X-55-	L-4/X-56-	L-4/X-57-	L-4/X-58-	L-4/X-59-	L-4/X-60-
	L-4/X-61-	L-4/X-62-	L-4/X-63-	L-4/X-64-	L-4/X-65-	L-4/X-66-
	L-4/X-67-	L-4/X-68-	L-4/X-69-	L-4/X-70-	L-4/X-71-	L-4/X-72-
	L-4/X-73-	L-4/X-74-	L-4/X-75-	L-4/X-76-	L-4/X-77-	L-4/X-78-
	L-4/X-79-	L-4/X-80-	L-4/X-81-	L-4/X-82-	L-4/X-83-	L-4/X-84-
15	L-4/X-85-	L-4/X-86-	L-4/X-87-	L-4/X-88-	L-4/X-89-	L-4/X-90-
	L-4/X-91-	L-4/X-92-	L-4/X-93-	L-4/X-94-	L-4/X-95-	L-4/X-96-
	L-4/X-97-	L-4/X-98-	L-4/X-99-	L-4/X-100-	L-4/X-101-	L-4/X-102-
	L-4/X-103-	L-4/X-104-	L-4/X-105-	L-4/X-106-	L-4/X-107-	L-4/X-108-
	L-4/X-109-	L-4/X-110-	L-4/X-111-	L-4/X-112-	L-4/X-113-	L-4/X-114-
20	L-4/X-115-	L-4/X-116-	L-4/X-117-	L-4/X-118-	L-4/X-119-	L-4/X-120-
	L-4/X-121-	L-4/X-122-	L-4/X-123-	L-4/X-124-	L-4/X-125-	L-4/X-126-
	L-4/X-127-	L-4/X-128-	L-4/X-129-	L-4/X-130-	L-4/X-131-	L-4/X-132-
	L-4/X-133-	L-4/X-134-	L-4/X-135-	L-4/X-136-	L-4/X-137-	L-4/X-138-
	L-4/X-139-	L-4/X-140-	L-4/X-141-	L-4/X-142-	L-4/X-143-	L-4/X-144-
25	L-4/X-145-	L-4/X-146-	L-4/X-147-	L-4/X-148-	L-4/X-149-	L-4/X-150-
	L-4/X-151-	L-4/X-152-	L-4/X-153-	L-4/X-154-	L-4/X-155-	L-4/X-156-
	L-4/X-157-	L-4/X-158-	L-4/X-159-	L-4/X-160-	L-4/X-161-	L-4/X-162-



	L-4/X-163	L-4/X-164	L-4/X-165	L-4/X-166	L-4/X-167	L-4/X-168
	L-4/X-169	L-4/X-170	L-4/X-171	L-4/X-172	L-4/X-173-	L-4/X-174-
	L-4/X-175-	L-4/X-176-	L-4/X-177-	L-4/X-178-	L-4/X-179-	L-4/X-180-
	L-4/X-181-	L-4/X-182-	L-4/X-183-	L-4/X-184-	L-4/X-185-	L-4/X-186-
5	L-4/X-187-	L-4/X-188-	L-4/X-189-	L-4/X-190-	L-4/X-191-	L-4/X-192-
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	L-4/X-211-	L-4/X-212-	L-4/X-213-	L-4/X-214-	L-4/X-215-	L-4/X-216-
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	L-4/X-223-	L-4/X-224-	L-4/X-225-	L-4/X-226-	L-4/X-227-	L-4/X-228-
	L-4/X-229-	L-4/X-230-	L-4/X-231-	L-4/X-232-	L-4/X-233-	L-4/X-234-
	L-4/X-235-	L-4/X-236-	L-4/X-237-	L-4/X-238-	L-4/X-239-	L-4/X-240-
	L-4/X-241-	L-4/X-242-	L-4/X-243-	L-4/X-244-	L-4/X-245-	L-4/X-246-
15	L-4/X-247-	L-4/X-248-	L-4/X-249-	L-4/X-250-	L-4/X-251-	L-4/X-252-
	L-4/X-253-	L-4/X-254-	L-4/X-255-	L-4/X-256-	L-4/X-257-	L-4/X-258-
	L-4/X-259-	L-4/X-260-	L-4/X-261-	L-4/X-262-	L-4/X-263-	L-4/X-264-
	L-4/X-265-	L-4/X-266-	L-4/X-267-	L-4/X-268-	L-4/X-269-	L-4/X-270-
	L-4/X-271-	L-4/X-272-	L-4/X-273-	L-4/X-274-	L-4/X-275-	L-4/X-276-
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	L-4/X-283-	L-4/X-284-	L-4/X-285-	L-4/X-286-	L-4/X-287-	L-4/X-288-
	L-4/X-289-	L-4/X-290-	L-4/X-291-	L-4/X-292-	L-4/X-293-	L-4/X-294-
	L-4/X-295-	L-4/X-296-	L-4/X-297-	L-4/X-298-	L-4/X-299-	L-4/X-300-
	L-4/X-301-	L-4/X-302-	L-4/X-303-	L-4/X-304-	L-4/X-305-	L-4/X-306-
25	L-4/X-307-	L-4/X-308-	L-4/X-309-	L-4/X-310-	L-4/X-311-	L-4/X-312-
	L-4/X-313-	L-4/X-314-	L-4/X-315-	L-4/X-316-	L-4/X-317-	L-4/X-318-
	L-4/X-319-	L-4/X-320-	L-4/X-321-	L-4/X-322-	L-4/X-323-	L-4/X-324-
	L-4/X-325-	L-4/X-326-	L-4/X-327-	L-4/X-328-	L-4/X-329-	L-4/X-330-
	L-4/X-331-	L-4/X-332-	L-4/X-333-	L-4/X-334-	L-4/X-335-	L-4/X-336-

L-4/X-337- L-4/X-338- L-4/X-339- L-4/X-340- L-4/X-341- L-4/X-342-  
 L-4/X-343- L-4/X-344- L-4/X-345- L-4/X-346- L-4/X-347- L-4/X-348-  
 L-4/X-349- L-4/X-350- L-4/X-351- L-4/X-352- L-4/X-353- L-4/X-354-  
 L-4/X-355- L-4/X-356- L-4/X-357- L-4/X-358- L-4/X-359- L-4/X-360-  
 5 L-4/X-361- L-4/X-362- L-4/X-363- L-4/X-364- L-4/X-365- L-4/X-366-  
 L-4/X-367- L-4/X-368- L-4/X-369- L-4/X-370- L-4/X-371- L-4/X-372-  
 L-4/X-373- L-4/X-374- L-4/X-375- L-4/X-376- L-4/X-377- L-4/X-378-  
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 10 L-4/X-391- L-4/X-392- L-4/X-393- L-4/X-394- L-4/X-395- L-4/X-396-  
 L-4/X-397- L-4/X-398- L-4/X-399- L-4/X-400- L-4/X-401- L-4/X-402-  
 L-4/X-403- L-4/X-404- L-4/X-405- L-4/X-406- L-4/X-407- L-4/X-408-  
 L-4/X-409- L-4/X-410- L-4/X-411- L-4/X-412- L-4/X-413- L-4/X-414-  
 L-4/X-415- L-4/X-416- L-4/X-417- L-4/X-418-

15

#### Pharmaceutical Formulations

When employed as pharmaceuticals, the compounds of Formula I are usually  
 administered in the form of pharmaceutical compositions. This invention therefore provides  
 pharmaceutical compositions which contain, as the active ingredient, one or more of the  
 20 compounds of Formula I above or a pharmaceutically acceptable salt thereof and one or more  
 pharmaceutically acceptable excipients, carriers, diluents, permeation enhancers, solubilizers  
 and adjuvants. The compounds may be administered alone or in combination with other  
 therapeutic agents (e.g., other antihypertensive drugs, diuretics and the like). Such  
 compositions are prepared in a manner well known in the pharmaceutical art (*see, e.g.,*  
 25 *Remington's Pharm. Sci.*, Mack Publishing Co., Philadelphia, PA, 17<sup>th</sup> Ed. (1985) and  
*"Modern Pharm."*, Marcel Dekker, Inc., 3<sup>rd</sup> Ed. (G.S. Banker & C.T. Rhodes, Eds.).

The compounds of Formula I may be administered by any of the accepted modes of  
 administration of agents having similar utilities, for example, by oral, parenteral, rectal,

buccal, intranasal or transdermal routes. The most suitable route will depend on the nature and severity of the condition being treated. Oral administration is a preferred route for the compounds of this invention. In making the compositions of this invention, the active ingredient is usually diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders. Pharmaceutically acceptable salts of the active agents may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, e.g., by J. March, *Advanced Organic Chem. Reactions, Mechanisms and Structure*, 4<sup>th</sup> Ed. (N.Y.: Wiley-Interscience, 1992).

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents.

The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art. Controlled release drug delivery systems for oral administration include osmotic pump systems and dissolutional systems containing polymer-coated reservoirs or drug-polymer matrix formulations. Examples of controlled release systems are given in U.S. Patent Nos. 3,845,770; 4,326,525; 4,902,514; and 5,616,345. Another preferred

formulation for use in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. *See, e.g.*, U.S. Patent Nos. 5,023,252; 4,992,445 and 5,001,139. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

The compositions are preferably formulated in a unit dosage form. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient (e.g., a tablet, capsule, ampoule). The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. Preferably, for oral administration, each dosage unit contains from 1-250 mg of a compound of Formula I, and for parenteral administration, preferably from 0.1 to 60 mg of a compound of Formula I or a pharmaceutically acceptable salt thereof. It will be understood, however, that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules.

5 The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

10 The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

15 Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

25 The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

5	<u>Ingredient</u>	Quantity
		<u>(mg/capsule)</u>
	Active Ingredient	30.0
	Starch	305.0
	Magnesium stearate	5.0

10 The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

15	<u>Ingredient</u>	Quantity
		<u>(mg/tablet)</u>
	Active Ingredient	25.0
	Cellulose, microcrystalline	200.0
20	Colloidal silicon dioxide	10.0
	Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

25 Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

<u>Ingredient</u>	<u>Weight %</u>
Active Ingredient	5
Lactose	95

5           The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

#### Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

10

<u>Ingredient</u>	<u>Quantity (mg/tablet)</u>
Active Ingredient	30.0
Starch	45.0
15   Microcrystalline cellulose	35.0
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0
Sodium carboxymethyl starch	4.5
Magnesium stearate	0.5
Talc	1.0
20   Total	120.0

25

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50°C to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

5	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
	Active Ingredient	40.0
	Starch	109.0
	Magnesium stearate	1.0
	Total	150.0

10

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

15      Suppositories, each containing 25 mg of active ingredient are made as follows:

<u>Ingredient</u>	<u>Amount</u>
Active Ingredient	25.0 mg
Saturated fatty acid glycerides to	2,000.0 mg

20

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

25

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:



<u>Ingredient</u>	<u>Amount</u>
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
5 Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 ml

10

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then

15 added to produce the required volume.

#### Formulation Example 8

<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
20 Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
25 Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

### Formulation Example 9

A subcutaneous formulation may be prepared as follows:

	<u>Ingredient</u>	<u>Quantity</u>
5	Active Ingredient	5.0 mg
	Corn Oil	1.0 mL

10 Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472 which is herein incorporated by reference.

15 Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the  
20 delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

### Synthesis Examples

25 Example 1. (Figure 7A)

Preparation of N,N'-dimethyl-N,N'-di-[2-[4-[2-butyl-3-benzofuranylcarbonyl]-2,6-diiodophenoxy]ethyl]hexane, (7A-2), in which n=1, and Link is (CH<sub>2</sub>)<sub>6</sub>.

5 A. A solution of 3-[(2-bromoethoxy)-3,5-diodobenzoyl]-2-butylbenzofuran (7A-1), prepared as described in *Eur. J. Med. Chem.*, 1974, 19-25, and in Belgian Patent 900138, (2 mmol), diisopropylethylamine (5 mmol) and 1,6-di-(methylamino)hexane (1 mmol) in acetonitrile (25mL) is maintained at room temperature, and the reaction is monitored by thin layer chromatography (tlc). When it is complete, the solution is added to water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 7A-2.

10 B. In a similar manner, by employing different diamines in place of 1,6-di-(methylamino)hexane, as described herein, in A above, different compounds of Formula 7A-2 are obtained.

15 C. In similar manner, by employing different bromo compounds of Formula 7A-1, as described herein, in A above, different compounds of Formula 7A-2 are obtained.

#### Example 2. (Figure 7B)

Preparation of 1,8-di-[2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diodophenoxy]ethyl]methylamino]-3,5-dioxaoctane, 7B-2, in which n is 1, and Link is  $(\text{CH}_2)_1(\text{O}(\text{CH}_2)_2)_2$ .

20 A. A solution of N-methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diodophenoxy]-ethylamine (7B-1), prepared according to procedures described in *Eur. J. Med. Chem.*, 1974, 19-25, (5 mmol), 1,8-dibromo-3,5-dioxaoctane (2.5 mmol) and diisopropylethylamine (2mL) in EtOH (25mL) is maintained at room temperature. The progress of the reaction is followed by tlc. When it is complete, the mixture is poured into water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 7B-2, in which n is 1 and Link is  $(\text{CH}_2)_2(\text{O}(\text{CH}_2)_2)_2$ .

B. In a similar manner, by employing different compounds 7B-1, as described herein, different linked compounds of Formula 7B-2 are obtained.

5 C. In a similar manner, by employing different linking compounds, as described herein, in place of 1,8-dibromo-3,5-dioxaoctane, different linked compounds of Formula 7B-2 are obtained.

### Example 3. (Figure 7C)

10 Preparation of 1,10-di-[2-butyl-[3-[4-(3-dibutylaminopropoxy)benzoyl]benzofuran-5-yl]aminosulfonyl]decane, 7C-2, in which Link is  $((CH_2)_2)_{10}$ .

15 A. 5-Amino-2-butyl-3-[4-(3-dibutylaminopropoxy)benzoyl]benzofuran (7C-1), prepared as described in EP 0471609, (1 mmol) and 1,10-di(chlorosulfonyl)decane (0.5 mmol) are heated at reflux in  $CH_2Cl_2$  (20mL). The progress of the reaction is followed by tlc. When it is complete, the solution is added to dilute  $Na_2CO_3$ . The organic phase is separated, dried and evaporated, and the residue is chromatographed to afford the title compound 7C-2.

20 B. In a similar manner, by employing different di-(chlorosulfonyl) linking compounds, as described herein, different linked compounds of Formula 7C-2 are obtained

### Example 4. (Figure 8A)

Preparation of 1,6-di[4-[2-[2-[4-(methylsulfonylamino)phenoxy]-ethylmethylamino]ethyl]phenylaminosulfonyl]hexane 8A-2, in which Link is  $(CH_2)_4$ .

25 A. 2-[4-(Methylsulfonylamino)phenoxy]ethyl bromide, 21-1, (10 mmol) and N-methyl 2-(4-nitrophenyl)ethylamine, 21-2, (10 mmol) both prepared as described in *J. Med. Chem.*, 1990, 1151, are heated at reflux in MeCN (100mL) containing  $K_2CO_3$  (3g) and KI (0.2g). The reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is

chromatographed to afford N-methyl N-(4-nitrophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]ethylamine (21-3).

5        B.     The above compound (3mmol) is dissolved in EtOH (50mL) and Raney nickel (1g) is added. The mixture is stirred in a hydrogen atmosphere. The progress of the reaction is monitored by tlc. When it is complete, the solution is filtered and then evaporated. The residue is chromatographed to afford N-methyl N-(4-aminophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]ethylamine, 8A-1.

10        C.     A solution of hexane-1,6-disulfonyl chloride (1 mmol), diisopropylethylamine (1mL) and 8A-1 (0.5 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (25mL) is maintained at room temperature. The progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under reduced pressure and the residue is chromatographed to afford the title compound 8A-2, in which Link is  $(\text{CH}_2)_4$ .

15        D.     In a similar manner, by employing different di(chlorosulfonyl) linking compounds, as described herein, in C above, different linked compounds 8A-2 are prepared.

#### Example 5. (Figure 8B)

20        Preparation of 1,4-di[-4-[2-methyl-2-[4-(methylsulfonylamino)phenyl]ethylamino]-ethoxy]phenyl]aminosulfonylmethyl]benzene, 8B-2, where Link is  $p\text{-CH}_2\text{C}_6\text{H}_4\text{CH}_2$ .

25        A.     2-(4-Nitrophenoxy)ethyl bromide, 21-4, (10 mmol) and 2-(4-methylsulfonylamino)phenyl N-methylethylamine 21-5 (10 mmol) are heated at reflux in MeCN (50mL) containing  $\text{K}_2\text{CO}_3$  (2g). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford N-methyl N-(4-(methylsulfonyl-amino)phenylethyl) 2-(4-aminophenoxy)ethylamine 8B-1.

B. The above compound **8B-1**, (1 mmol) and 1,4-di-(chlorosulfonylmethyl)benzene (0.5 mmol) are dissolved in  $\text{CH}_2\text{Cl}_2$  (50 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is washed with dilute  $\text{Na}_2\text{CO}_3$ , then dried and evaporated. The residue is chromatographed to afford **8B-2**, in which  
5 Link is  $\text{p-CH}_2\text{C}_6\text{H}_4\text{CH}_2$ .

C. In a similar manner, by employing different di(sulfonyl chloride) linking compounds, as described herein, in B above, different linked compounds **8B-2** are prepared

10 Example 6. (Figure 8C)

Preparation of 1,10-di-[2-[4-(methylsulfonylaminophenoxy)ethyl] 2-[4-[methylsulfonylaminophenyl]ethyl]amino]decane, **8C-2**, in which Link is  $(\text{CH}_2)_{10}$ .

A. N-Benzyl 2-[4-(methylsulfonylaminophenyl)]ethylamine, (**21-7** prepared as  
15 described in EP 338793), (10 mmol), 2-[4-(methylsulfonylaminophenoxy)ethyl bromide **21-8** (10 mmol) and  $\text{K}_2\text{CO}_3$  (1 g) are heated at reflux in MeCN (50 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford N-benzyl N-2-(4-methylsulfonylaminophenoxy)ethyl 2-(4-  
20 methylsulfonylaminophenyl)ethylamine, **21-9**.

B. The compound **21-9** (1 mmol) is dissolved in EtOH (20 mL) and ammonium formate (100 mg) and 10% Pd/C (50 mg) are added. The progress of the reaction is monitored by tlc. When it is complete, the solution is filtered then added to water and  
25 extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford N-[2-(4-methylsulfonylaminophenoxy)ethyl] 2-(4-methylsulfonylaminophenyl)-ethylamine, **8C-1**.

C. The above compound (1mmol), 1,10-dibromodecane (0.5 mmol),  $K_2CO_3$  (1g) and KI (0.05g) are heated at reflux in MeCN. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated. The residue is chromatographed to afford the title compound 8C-2, in which Link is  $(CH_2)_{10}$ .

D. In a similar manner, by employing different dialkylating agents, as described herein, in place of 1,10-dibromodecane, in C above, different compounds of Formula 8C-2 are obtained.

#### Example 7. (Figure 9A)

**Preparation of 1,8-di-[4-[4-(ethylheptylamino)-1-hydroxybutyl]phenylamino-sulfonyl]octane, 9A-2, in which Link is  $(CH_2)_6$ .**

A. 4-Nitrophenyl-4-oxobutanoic acid, 21-10, prepared as described in *Gazz. Chim. Ital.*, 1967, 97, 654, and U.S. Patent 5,405,851 (10 mmol), 1-hydroxybenztriazole (10 mmol) and dicyclohexylcarbodiimide (10 mmol) are dissolved in DMF (50mL). To the solution is added ethylheptylamine (10 mmol). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford N-ethyl N-heptyl 4-(4-nitrophenyl)-4-oxobutanamide, 21-11.

B. The above prepared compound (2 mmol) is dissolved in dry diglyme (20mL) and MeOH (1mL). Lithium borohydride (25 mmol) is added and the solution is heated to reflux. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford 4-[1-hydroxy-4(ethylheptylamino)butyl]aniline 9A-1.

C. The compound 9A-1 (1 mmol) and octane-1,8-disulfonyl chloride (0.5 mmol) are dissolved in  $\text{CH}_2\text{Cl}_2$  (25mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute  $\text{Na}_2\text{CO}_3$ , and then extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 9A-2., in which Link is  $(\text{CH}_2)_6$ .

D. In a similar manner, by employing different disulfonyl chlorides, as described herein, in C above, different compounds of Formula 9A-2 are obtained.

#### Example 8. (Figure 9B)

Preparation of 1,6-di-[4-[4-hydroxy-4-(methylsulfonylaminophenyl)butyl]-ethylamino]hexane 9B-2, in which Link is  $(\text{CH}_2)_6$ .

A. 4-(4-Aminophenyl)-4-oxobutanoic acid 21-12, (5 mmol) is added to a solution of dicyclohexylcarbodiimide (5 mmol) and ethylamine (5 mmol) in THF (50mL). After 12 hours, the mixture is added to water and extracted with EtOAc. The extract is washed with dilute NaOH, then dried and evaporated. The residue is chromatographed to afford N-ethyl 4-(4-aminophenyl)-4-oxobutanamide, 21-13.

B. The product from A above (3 mmol) is dissolved in THF (25mL) and to the solution is added diisopropylethylamine (5 mmol) and methanesulfonyl chloride (3 mmol). The reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford N-ethyl 4-(4-methylsulfonylaminophenyl)-4-oxobutanamide, 21-14.

C. The above-described compound (1 mmol) is dissolved in ether (50mL); the solution is cooled to  $0^\circ\text{C}$ , and to it is added lithium aluminum hydride (5 mmol). The reaction is monitored by tlc. When it is complete, excess hydride is decomposed by addition



of aqueous potassium sodium tartrate. The organic phase is separated, dried and evaporated, and the residue is chromatographed to afford N-ethyl 4-(4-methylsulfonylamino-phenyl)-4-hydroxybutylamine, **9B-1**:

5           D.       The compound **9B-1** (1mmol), diisopropylethylamine (2 mmol) and 1,6-dibromohexane (0.5 mmol) are dissolved in MeCN (25 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute  $\text{Na}_2\text{CO}_3$ , and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to afford the title compound **9B-2**, in which Link is  $(\text{CH}_2)_6$ .

10           E.       In a similar manner, by employing different dialkylating agents, as described herein, in place of 1,6-dibromohexane, different compounds of Formula **9B-2** are obtained.

#### Example 9. (Figure 10A)

15       Preparation of 1,8-di-[4-[2-[diethylaminoethyl]aminocarbonyl]phenylamino-sulfonyl]octane, **10A-2**, in which Link is  $(\text{CH}_2)_6$ .

20           A.       Procaine amide (**10A-1**) (10 mmol) is dissolved in MeCN (50 mL) and 1,8-di-(chlorosulfonyl)octane (5 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute  $\text{Na}_2\text{CO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated to afford the title compound, **10A-2**, in which Link is  $(\text{CH}_2)_6$ .

25           B.       In a similar manner, by employing different disulfonyl chlorides, as described herein, different compounds of Formula **10A-2** are obtained.

#### Example 10. (Figure 10B)

Preparation of 1,8-di-[N-ethyl N'-[2-[4-[methylsulfonylamino]benzoylaminoethyl]-amino] 3,5-dioxaoctane, **10B-2**, in which Link is  $(\text{CH}_2)_2(\text{O}(\text{CH}_2)_2)_2$ .

A. N-Ethyl N'-(4-methylsulfonylaminobenzoyl)ethylenediamine, (10B-1), prepared as described in *J. Med. Chem.*, 1987, 30, 755, (5 mmol) diisopropylethylamine (5 mmol) and 1,8-dibromo-3,5-dioxaoctane (2.5 mmol) are dissolved in MeCN (25 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute  $\text{Na}_2\text{CO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$ . The solution is dried and evaporated, and the residue is chromatographed to afford the title compound 10B-2, in which Link is  $(\text{CH}_2)_2(\text{O}(\text{CH}_2)_2)_2$ .

B. In a similar manner, by employing different dialkylating agents, as described herein, different compounds of Formula 10B-2 are obtained.

#### Example 11. (Figure 11)

**Preparation of 1,10-di-[2-hydroxy-2-[4-methylsulfonylaminophenyl]ethylamino]-decane, 11-2, in which Link is  $(\text{CH}_2)_{10}$ .**

A. 1,10-Dibromodecane (5mmol), 2-hydroxy-2-(4-methylsulfonylaminophenyl)-ethylamine (11-1) (10 mmol), prepared as described in European Patent 338793, potassium iodide (0.1g) and  $\text{K}_2\text{CO}_3$  (1g) are stirred in MeCN (50mL). The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 11-2, in which Link is  $(\text{CH}_2)_{10}$ .

B. In a similar manner, by employing different dialkylating agents, as described herein, different compounds of Formula 11-2 are obtained.

#### Example 12. (Figure 12)

**Preparation of 1,6-di[4-[1-[5-(4-chlorophenyl)-2-furanylmethylene]amino]-imidazolidine-2,4-dione-3-yl]butylmethylamino]hexane, 12-2, where Link is  $(\text{CH}_2)_6$ .**

A. 1-Benzylamino-3-(4-iodobutyl)imidazolidine-2,4-dione (12-5), prepared as described in W093/04061, (5 mmol) is added to a solution of methylamine (2g) in MeOH (40mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated and the  
5 residue is chromatographed to afford 1-benzylamino-3-(4-methylaminobutyl)imidazolin-2,4-dione, 12-6.

B. The latter compound 12-6, (2 mmol) is added to EtOH (25mL) containing 10% Pd/C (50mg) and ammonium formate (0.5g). The progress of the reaction is followed by tlc.  
10 When it is complete, the solution is filtered and added to water. The aqueous solution is extracted with EtOAc. The extract is dried and evaporated. The residue is chromatographed to afford 1-amino-3-(4-methylaminobutyl)imidazolidine-2,4-dione, 12-7.

C. The above-described compound (1 mmol) is dissolved in EtOH (20mL). To  
15 the solution is added 5-(4-chlorophenyl)furan-2-carboxaldehyde (12-8), (1 mmol) and p-toluenesulfonic acid (10mg). The progress of the reaction is followed by tlc. When it is complete, the mixture is added to water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated to afford 1-[5-(4-chlorophenyl)-2-furanylmethyleneamino]-3-[4-(methylamino)butyl]imidazolidine-2,4-dione, 12-1.

D. A solution of 12-1 (1 mmol), 1,6-di-(p-toluenesulfonyloxy)hexane (0.5 mmol) and diisopropylethylamine (3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) is heated at reflux. The progress of the reaction is monitored by tlc. When it is complete, the solution is cooled and added to water. The aqueous solution is extracted with CH<sub>2</sub>Cl<sub>2</sub>, and the extract is dried and  
25 evaporated. The residue is chromatographed to afford the title compound 12-2, in which Link is (CH<sub>2</sub>)<sub>6</sub>.

E. In a similar manner, by employing other dialkylating agents, as described herein, different compounds of Formula 12-2 are obtained.

## Example 13. (Figure 12)

Preparation of 1,4-di[4-[1-[[5-(4-chlorophenyl)-2-furanylmethylene]amino]-imidazolidine-2,4-dione-3-yl]4-butyl(piperazin-1-yl)]butane, 12-4, where Link is  $(\text{CH}_2)_4$ .

5           A.     1-Benzylamino-3-(4-iodobutyl)imidazolidine-2,4-dione (12-5), prepared as described in W093/04061, (5 mmol), diisopropylethylamine (10 mmol) and N-benzyloxycarbonylpiperazine (5 mmol) are dissolved in MeCN (50 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to  
10           afford 1-benzylamino-3-[4-(4-benzyloxycarbonylpiperazinyl)butyl]-imidazoline-2,4-dione, 12-9.

          B.     The above compound, (2 mmol) is dissolved in EtOH (25 mL), and to the solution are added 5% Pd/C (100mg) and ammonium formate (250mg). The progress of the  
15           reaction is monitored by tlc. When it is complete, the mixture is filtered and added to water, then extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford 1-amino-3-[4-(piperazin-1-yl)butyl]imidazoline-2,4-dione, 12-10.

          C.     The above-described compound (1 mmol) is dissolved in EtOH (20mL). To  
20           the solution is added 5-(4-chlorophenyl)furan-2-carboxaldehyde (12-8), (1 mmol) and p-toluenesulfonic acid (10mg). The progress of the reaction is followed by tlc. When it is complete, the mixture is added to water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated to afford 1-[5-(4-chlorophenyl)-2-furanylmethyleneamino]-3-[4-(piperazin-1-yl)butyl]imidazolidine-2,4-dione, 12-3.

25           D.     A solution of 1,4-dibromobutane, (0.5 mmol) and 12-3 (1 mmol) in EtOH is maintained at room temperature, while the progress of the reaction is monitored by tlc. When it is complete, the mixture is evaporated to dryness under reduced pressure, and the residue is chromatographed to afford 12-4, in which Link is  $(\text{CH}_2)_4$ .

E. In a similar manner, by employing different dialkylating agents, as described herein, in part D above, different compounds of Formula 12-4 are obtained.

Example 14. (Figure 13)

5 Preparation of 1,10-di-(3-cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo-[3.3.1]non-7-yl)decane, 13-2, in which Link is  $(CH_2)_{10}$ .

A. 3-Benzyl-7-cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, (20-1, in which  $R_1$  is benzyl), the preparation of which is described in European Patent 461574, (Table 1, compound 22), (5 mmol) is dissolved in MeOH (20 mL) containing 5% Pd/C (50mg) and formic acid (0.5 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is filtered and the solvent is removed under reduced pressure. The residue is chromatographed to afford 3-cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, (20-1, in which  $R_1$  is H).  
15 H).

B. 1,10-Dibromodecane (0.5 mmol) is added to a solution of the compound 20-1 in which  $R_1$  is H, prepared as described above, (1 mmol) and diisopropylethylamine (0.5 mL) in DMF (10 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute  $Na_2CO_3$  and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 13-2, in which Link is  $(CH_2)_{10}$ .  
20

C. In a similar manner, by employing different dialkylating agents, as described herein, different compounds of structure 13-2 are obtained.  
25

## Example 15. (Figure 14)

Preparation of 1,4-di-[2-[2-[4-(2-butylbenzofuran-3-yl)carbonyl-2,6-diiodophenoxyethyl]methylamino]acetylaminobutane, 14-2, in which n is 1 and Link is (CH<sub>2</sub>)<sub>4</sub>.

5

A. The compound 7B-1, prepared according to procedures described in *Eur. J. Med. Chem.*, 1974, 19-25, (5 mmol), diisopropylethylamine (5 mmol) and ethyl bromoacetate (5 mmol) are dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated; the residue is chromatographed to afford ethyl N-methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylaminoacetate, 14-1, in which R is ethyl.

10

B. The above compound (1 mmol) is dissolved in THF (10 mL) and water (3 mL), and lithium hydroxide monohydrate (1.25 mmol) is added. The reaction is monitored by tlc. When it is complete, acetic acid (2 mmol) is added, and the solvents are removed under reduced pressure. The residue is chromatographed to afford N-methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylaminoacetic acid, 14-1, in which R is H.

15

C. The above-prepared compound (1 mmol) is dissolved in DMF (20 mL) and dicyclohexylcarbodiimide (1 mmol) and 1,4-diaminobutane (0.5 mmol) are added. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated; the residue is chromatographed to afford the title compound 14-2, in which n is 1 and Link is (CH<sub>2</sub>)<sub>4</sub>.

20

25

D. In a similar manner, by employing different diamines, as described herein, different compounds of Formula 14-2 are obtained.

## Example 16. (Figure 14)

Preparation of 1,4-di-[3-[4-[2-[4-(methylsulfonylamino)phenoxy]ethyl]-aminoethyl]phenylaminosulfonyl]propylmethylamino]butane, 14-7, in which Link is  $(CH_2)_4$ .

5

A. N-methyl N-(4-aminophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]ethylamine, 14-3, prepared as described in Examples 3A and 3B, (5 mmol) is dissolved in  $CH_2Cl_2$  (25 mL) and 3-azidopropylsulfonylchloride (5 mmol) is added. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated; the residue is chromatographed to afford N-methyl N-[4-(3-azidopropylsulfonyl)aminophenylethyl] 2-[4-(methylsulfonylamino)phenoxy]-ethylamine, 14-5.

10

B. The above-prepared compound (1 mmol) is dissolved in MeOH (20 mL) and 5% Pd/C (50 mg) is added. The mixture is stirred in a hydrogen atmosphere. The progress of the reaction is followed by tlc. When it is complete, the solution is filtered and the solvent is removed under reduced pressure. The residue is redissolved in MeOH (20 mL) and paraformaldehyde (1 mmol) and sodium cyanoborohydride (1 mmol) are added. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford N-methyl N-[4-(3-methylaminopropylsulfonyl)-aminophenylethyl]-2-[4-(methylsulfonylamino)phenoxy]ethylamine, 14-6.

15

20

C. 1,4-Dibromobutane (0.5 mmol) is dissolved in MeCN, and  $K_2CO_3$  (0.5 g) and the compound 14-6 (0.25 mmol) are added. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 14-7, in which Link is  $(CH_2)_4$ .

25

D. In a similar manner, by employing different dialkylating agents, as described herein, in step C above, different compounds of Formula 14-7 are obtained.

Example 17. (Figure 14)

5 Preparation of 1,8-di-[[N-[2-(4-methylsulfonylaminophenoxy)ethyl] N-2-(4-methylsulfonylaminophenyl)ethyl] 2-aminoethoxy]octane, 14-10, in which Link is  $(\text{CH}_2)_8$ .

10 A. N-2-(4-Methylsulfonylaminophenoxy)ethyl 2-(4-methylsulfonylaminophenyl)-ethylamine, (14-8), the preparation of which is described above in Examples 6A and 6B, (1 mmol) is dissolved in EtOH (20 mL) and to the solution is added 2-bromoethanol (1 mmol) and diisopropylethylamine (5 mmol). The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford N-(2-hydroxyethyl) N-[2-(4-

15 methylsulfonylaminophenoxy)ethyl] 2-(4-methylsulfonylaminophenyl)ethylamine, 14-9.

B. The compound 14-9, (1 mmol) is dissolved in DMSO (10 mL) and KOH (10 mmol) and 1,8-dibromooctane (0.5 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with

20 EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound, 14-10, in which Link is  $(\text{CH}_2)_8$ .

C. In a similar manner, by employing different dihalo compounds, in place of 1,8-dibromooctane, different compounds of Formula 14-10 can be obtained.

25 Example 18. (Figure 15)

Preparation of 1,4,8,12-tetra-[2-(4-methylsulfonylaminophenoxy)ethyl]-1,4,8,12-tetraazacyclohexadecane, 15-3, in which X is O.



A. 2-(4-Methylsulfonylamino)phenoxyethyl bromide (15-1, in which X is O), the preparation of which is described in *J. Med. Chem.*, 1990, 1551, (4 mmol) and 1,4,8,12-tetraazacyclohexadecane (15-2) (1 mmol) are dissolved in MeCN (25 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and  
5 extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound, 15-3, in which X is O.

B. In a similar manner, by employing 2-(4-methylsulfonylamino)phenyl)ethylamine, (15-1, in which X is a direct bond, the preparation of which is described in *J. Med. Chem.*, 1990, 1551), there is obtained 1,4,8,12-tetra-[2-(4-methylsulfonylamino)phenyl)ethyl]-1,4,8,12-tetraazacyclohexadecane, 15-3, in which X is a  
10 direct bond.

#### Example 19. (Figure 15)

15 Preparation of 1,3,5-tri-[N-methyl 2-[2,6-diiodo-4-[2-butyl-3-benzofuran-ylcarbonyl]phenoxy]ethyl]aminomethyl]benzene, 15-6.

N-Methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylamine (15-4), prepared according to procedures described in *Eur. J. Med. Chem.*, 1974, 19-25, (3 mmol)  
20 is dissolved in MeCN (30 mL), and 1,3,5-tri(bromomethyl)benzene (1 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.5g) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 15-6.

#### Example 20. (Figure 15)

25 Preparation of the trimeric amide 15-9.

A. N-Methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylamine (15-4), prepared according to procedures described in *Eur. J.*

*Med. Chem.*, 1974, 19-25, (5 mmol) is dissolved in EtOH (25 mL) and ethyl bromoacetate (5 mmol) and diisopropylethylamine (10 mmol) are added. The progress of the reaction is followed by tlc. When it is complete, the reaction is added to water and extracted with EtOAc. The extract is washed with dilute HCl, the dried and the solvent is evaporated under reduced pressure. The residue is dissolved in THF (15 mL), and LiOH, H<sub>2</sub>O (5 mmol) is added. The progress of the reaction is followed by tlc. When it is complete, the pH is adjusted to 7 by addition of dilute HCl. The solvents are removed under reduced pressure and the residue is chromatographed to afford N-methyl N-[2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diodophenoxy]ethyl]glycine, 15-7.

B. The compound 15-7 (3 mmol) is dissolved in DMF (25 mL) and dicyclohexylcarbodiimide (3 mmol) and tris(2-aminoethyl)amine (1 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the triamide product 15-9.

#### Example 21. (Figure 15)

Preparation of 1,4-di-[N-methyl 2-(4-methylsulfonylaminophenoxy)-ethylamino]butane, 15-12, in which X is O, R is methyl and Link is (CH<sub>2</sub>)<sub>4</sub>.

A. 2-(4-Methylsulfonylaminophenoxy)ethyl bromide, 15-10, in which X is O, (2 mmol) and 1,4-di(methylamino)butane, 15-11, (1 mmol) are dissolved in MeCN (20 mL) containing K<sub>2</sub>CO<sub>3</sub> (0.5g). The progress of the reaction is followed by tlc. When it is complete, the reaction is added to water and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 15-12.

B. In a similar manner, by employing 2-(4-methylsulfonylaminophenyl)ethyl bromide in A above, there is obtained the corresponding product 1,4-di-[N-methyl 2-(4-

methylsulfonylaminophenyl)-ethylamino]butane, 15-12, in which X is a direct bond, R is methyl and Link is  $(CH_2)_4$ .

C. In a similar manner, by employing different diamines 15-11, as described herein, in A and B above, there are obtained the corresponding diamine products 15-12.

#### Example 22. (Figure 18)

Preparation of the asymmetrically linked aminosulfonamide 18-4, in which Link is  $(CH_2)_7$ .

A. N-Methyl N-(4-aminophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]-ethylamine, 18-1, the preparation of which is described in Examples 4A and 4B above, (2 mmol) is dissolved in dry  $CH_2Cl_2$  (25 mL); diisopropylethylamine (10 mmol) and 3-bromopropanesulfonyl chloride (2 mmol) are added. The progress of the reaction is followed by tlc. When it is complete, the reaction is added to water and extracted with EtOAc. The extract is washed and dried and the solvent is evaporated under reduced pressure. The residue is chromatographed to afford 1-bromo-3-[4-[N-methyl 2-[2-[4-methylsulfonylamino]phenoxy]ethylamino]phenylaminosulfonyl]propane, 18-2, in which Link is  $(CH_2)_2$ .

B. N 2-(4-aminophenyl)ethyl 2-[4-methylsulfonylaminophenoxy]ethylamine, 18-3, prepared using methods described in EP 338793, (1 mmol) and the compound 18-2, (1 mmol) are dissolved in  $CH_2Cl_2$  (25 mL) and to the solution is added diisopropylethylamine (10 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 18-4, in which Link is  $(CH_2)_2$ .

C. In a similar manner, by employing different bromosulfonyl chlorides, as described herein, the corresponding compounds of Formula 18-4 are obtained.

## Example 23. (Figure 19)

Preparation of the dimeric heterovalomer 19-4, in which Link is  $(CH_2)_5$ .

A. Using the procedure described in Example 22A, but using 6-bromohexanesulfonyl chloride instead of 3-bromopropanesulfonyl chloride, 1-bromo-6-[4-[N-methyl 2-[2-[4-methylsulfonylamino]phenoxy]ethylamino]phenylaminosulfonyl]hexane, 19-2, in which Link is  $(CH_2)_5$ , is prepared.

B. The above compound (2 mmol) and N-ethyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylamine (19-3), prepared according to procedures described in *Eur. J. Med. Chem.*, 1974, 19-25, are dissolved in MeCN (25 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute  $NaHCO_3$  and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 19-4, in which Link is  $(CH_2)_5$ .

C. In a similar manner, by employing different bromo compounds 19-2, the corresponding heterovalomers 19-4 are obtained.

## Example 24. (Figure 19)

Preparation of the dimeric heterovalomer 19-8, in which Link is  $(CH_2)_5$ .

A. N-[4-[[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl methanesulfonamide, (E-4031, Table 4) prepared as described in *J. Med. Chem.*, 1990, 903, (10 mmol) is dissolved in 48% HBr in AcOH (50 mL). The solution is heated to 60°C and the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and the solvent is removed under reduced pressure. The residue is taken up in water and the solution is basified with aqueous NaOH. The aqueous solution is extracted with  $CH_2Cl_2$ , and the extract is dried and evaporated. The residue is chromatographed to afford 6-[2-[4-[4-aminobenzoyl-1-piperidyl]ethyl]-2-methylpyridine, 19-5.

B. The above compound 19-5 (2 mmol) is dissolved in  $\text{CH}_2\text{Cl}_2$  (35 mL) and to the solution are added diisopropylethylamine (5 mmol) and 6-bromohexanesulfonyl chloride (2 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute  $\text{NaHCO}_3$  and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford 19-6, in which Link is  $(\text{CH}_2)_5$ .

C. To a solution of the above compound (1 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) is added N-[2-(4-methylsulfonylaminophenoxy)ethyl] 2-(4-methylsulfonylaminophenyl)-ethylamine, 19-7, the preparation of which is described in Example 6A and 6B, (1 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute  $\text{NaHCO}_3$  and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the dimeric product 19-8, in which Link is  $(\text{CH}_2)_5$ .

D. In a similar manner, by employing different bromoalkyl sulfonyl chlorides, as described herein, the corresponding products of Formula 19-8 are obtained.

#### Example 25. (Figure 19)

**Preparation of the dimeric heterovalomer 19-11, in which Link is  $(\text{CH}_2)_3$ .**

A. Using the procedure of Example 24A, except that 4-bromobutanesulfonyl chloride is employed instead of 6-bromohexanesulfonyl chloride, there is prepared the compound 19-9, in which Link is  $(\text{CH}_2)_3$ .

B. N-Methyl N-(4-aminophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]ethylamine, (8A-1, the preparation of which is described in Examples 4A and 4B) (2 mmol) is dissolved in MeCN (25 mL) and to the solution is added 3-bromopropanesulfonyl chloride (2 mmol). After 6 hours, 10% methanolic methylamine (1 mL) is added. The progress of the reaction is followed by tlc. When it is complete, the



product 3-cyclopropylmethyl 7-[2-[4-methylsulfonylaminophenyl]ethyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-3, in which X is a direct bond.

Example 27. (Figure 20)

5 Preparation of 3,8-di-[2-[4-methylsulfonylaminophenoxy]ethyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-5, in which X is O.

A. 3,8-Dibenzyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-4, in which R<sub>1</sub> and R<sub>2</sub> are benzyl, the preparation of which is described in European Patent 461 574, 10 (5 mmol) is dissolved in EtOH (25 mL). 10% Pd/C (50 mg) and ammonium formate (200 mg) are added. The progress of the reaction is followed by tlc. When it is complete, the solution is filtered and the solvent is removed under vacuum to afford 9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-4, in which R<sub>1</sub> and R<sub>2</sub> are H.

15 B. The above compound (1 mmol) is dissolved in MeCN, and to the solution is added diisopropylethylamine (5 mmol) and 2-[4-methylsulfonylaminophenoxy]ethyl bromide (0.5 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 20-5, in which X is O.

20 C. In a similar manner, by employing 2-[4-methylsulfonylaminophenyl]ethyl bromide in B above, there is obtained the corresponding product 3,8-di-[2-[4-methylsulfonylaminophenyl]ethyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane 20-5, in which X is a direct bond.

25

## Example 28. (Figure 20)

Preparation of 3-cyclopropylmethyl-7-[4-[2-[4-methylsulfonylamino]phenoxy]-ethylmethylamino]butyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-5, in which X is O.

5  
A. 3-Cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, (20-1, in which R is H), (5 mmol) and 1,4-dibromobutane (5 mmol) are dissolved in EtOH (30 mL). The progress of the reaction is followed by tlc. When it is complete, N-methyl 2-(4-methylsulfonylamino]phenoxy)ethylamine (20-6, in which X is O), (5 mmol) is added. The  
10 progress of the reaction is followed by tlc. When it is complete, the mixture is added to dilute NaHCO<sub>3</sub> and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 20-5, in which X is O.

15 B. In a similar manner, by employing 2-[4-methylsulfonylamino]phenyl]ethyl bromide in A above, there is obtained the corresponding product 3-cyclopropylmethyl-7-[4-[2-[4-methylsulfonylamino]phenyl]-ethylmethylamino]butyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-5, in which X is a direct bond.

20 C. In a similar manner, by employing different dibromo compounds in place of 1,4-dibromobutane, there are obtained the corresponding products similar to 20-7.

## Example 29. (Figure 20)

Preparation of 1,3,5-tri-[2-[4-(methylsulfonylamino)phenoxy]ethylmethylamino-methyl]benzene, 20-9, in which X is O.

25 A. N-Methyl 2-[(4-methylsulfonylamino)phenoxy]ethylamine (20-6, in which X is O), (3 mmol) and 1,3,5-tri-(bromomethyl) benzene (20-8), (1 mmol) are dissolved in MeCN (25 mL) containing K<sub>2</sub>CO<sub>3</sub> (0.5g). The progress of the reaction is monitored by tlc.



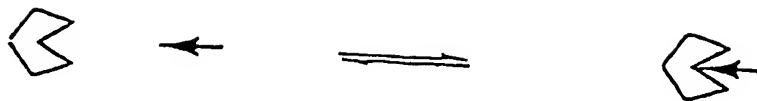
different binding affinities for different states, and be capable of producing agonist or antagonist activity.

5 The term "modulatory effect" is intended to refer to the ability of a ligand to change the activity of a K<sup>+</sup> channel through binding to the channel.

10 "Multibinding agent" or "multibinding compound" refers herein to a compound that has from 2 to 10 K<sup>+</sup> channel ligands as defined herein (which may be the same or different) covalently bound to one or more linkers (which may be the same or different), and is capable of multivalency, as defined below.

15 A multibinding compound provides an improved biologic and/or therapeutic effect compared to that of the same number of unlinked ligands available for binding to the ligand binding sites on a K<sup>+</sup> channel or channels. Examples of improved "biologic and/or therapeutic effect" include increased ligand-receptor binding interactions (e.g., increased affinity, increased ability to elicit a functional change in the target, improved kinetics), increased selectivity for the target, increased potency, increased efficacy, decreased toxicity, increased therapeutic index, improved duration of action, improved bioavailability, improved pharmacokinetics, improved activity spectrum, and the like. The multibinding compounds of this invention will exhibit at least one, and preferably more than one, of the above-mentioned effects.

25 "Univalency" as used herein refers to a single binding interaction between one ligand with one ligand binding site as defined herein. It should be noted that a compound having multiple copies of a ligand (or ligands) exhibits univalency when only one ligand of that compound interacts with a ligand binding site. Examples of univalent interactions are depicted below.



univalent interaction

5

"Multivalency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands, which may be the same or different, and two or more corresponding ligand binding sites, which may be the same or different. An example of trivalent binding is depicted below for illustrative purposes.

10



15

trivalent interaction

20

It should be understood that not all compounds that contain multiple copies of a ligand attached to a linker necessarily exhibit the phenomena of multivalency, i.e., that the biologic and/or therapeutic effect of the multibinding agent is greater than that of the same number of unlinked ligands made available for binding to the ligand binding sites. For multivalency to occur, the ligand domains of the ligands that are linked together must be presented to their cognate ligand binding sites by the linker or linkers in a specific manner in order to bring about the desired ligand-orienting result, and thus produce a multibinding interaction.

25

The term "library" refers to at least 3, preferably from  $10^2$  to  $10^9$  and more preferably from  $10^2$  to  $10^4$  multimeric compounds. Preferably, these compounds are prepared as a multiplicity of compounds in a single solution or reaction mixture which permits facile synthesis thereof. In one embodiment, the library of multimeric compounds can be directly assayed for multibinding properties. In another embodiment, each member of the library of

multimeric compounds is first isolated and, optionally, characterized. This member is then assayed for multibinding properties.

5 The term "collection" refers to a set of multimeric compounds which are prepared either sequentially or concurrently (e.g., combinatorially). The collection comprises at least 2 members; preferably from 2 to  $10^9$  members and still more preferably from 10 to  $10^4$  members.

10 The term "multimeric compound" refers to compounds comprising from 2 to 10 ligands covalently connected through at least one linker which compounds may or may not possess multibinding properties (as defined herein).

15 The term "pseudohalide" refers to functional groups which react in displacement reactions in a manner similar to a halogen. Such functional groups include, by way of example, mesyl, tosyl, azido and cyano groups.

20 The term "linker", identified where appropriate by the symbol X, refers to a group or groups that covalently links from 2 to 10 ligands (as defined above) in a manner that provides a compound capable of multivalency. The linker is a ligand-orienting entity that permits attachment of multiple copies of a ligand (which may be the same or different) thereto.

25 The term "linker" includes everything that is not considered to be part of the ligand, e.g., ancillary groups such as solubilizing groups, lipophilic groups, groups that alter pharmacodynamics or pharmacokinetics, groups that modify the diffusability of the multibinding compound, spacers that attach the ligand to the linker, groups that aid the ligand-orienting function of the linker, for example, by imparting flexibility or rigidity to the linker as a whole, or to a portion thereof, and so on. The term "linker" does not, however, cover solid inert supports such as beads, glass particles, rods, and the like, but it is to be understood that the multibinding compounds of this invention can be attached to a solid

understood that the multibinding compounds of this invention can be attached to a solid support if desired, for example, for use in separation and purification processes and for similar applications.

5           The extent to which the previously discussed enhanced activity of multibinding compounds is realized in this invention depends upon the efficiency with which the linker or linkers that joins the ligands presents them to their array of ligand binding sites. Beyond presenting these ligands for multivalent interactions with ligand binding sites, the linker spatially constrains these interactions to occur within dimensions defined by the linker.

10           The linkers used in this invention are selected to allow multivalent binding of ligands to any desired ligand binding sites of a  $K^+$  channel, whether such sites are located within the cell membrane, interiorly (e.g., within a channel/translocation pore), both interiorly and on the periphery of a channel, at the boundary region between the lipid bilayer and the channel, or at  
15 any intermediate position thereof. The preferred linker length will vary depending on the distance between adjacent ligand binding sites, and the geometry, flexibility and composition of the linker. The length of the linker will preferably be in the range of about 2Å to about 100Å, more preferably from about 2Å to about 50Å and even more preferably from about 5Å to about 20Å.

20           The ligands are covalently attached to the linker or linkers using conventional chemical techniques. The reaction chemistries resulting in such linkage are well known in the art and involve the use of reactive functional groups present on the linker and ligand. Preferably, the reactive functional groups on the linker are selected relative to the functional  
25 groups available on the ligand for coupling, or which can be introduced onto the ligand for this purpose. Again, such reactive functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable well-known activating agents results in formation of an amide bond covalently linking the ligand to the linker;

reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker. The table below and Figure 6 illustrate numerous reactive functional groups and the resulting bonds formed by reaction therebetween. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as J. March, *Advanced Organic Chemistry*, 4<sup>th</sup> Ed., (Wiley-Interscience, N.Y., 1992).

Complementary Binding Chemistries

First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	$\beta$ -hydroxyamine
sulfonyl halide	amine	sulfonamide
carboxyl	amine	amide
hydroxyl	alkyl/aryl halide	ether
amine	alkyl halide	substituted amine

The linker is attached to the ligand at a position that retains ligand domain-ligand binding site interaction and specifically which permits the ligand domain of the ligand to orient itself to bind to the ligand binding site. Such positions and synthetic protocols for linkage are well known in the art. The term linker embraces everything that is not considered to be part of the ligand.

The relative orientation in which the ligand domains are displayed depends both on the particular point or points of attachment of the ligands to the linker, and on the framework geometry. The determination of where acceptable substitutions can be made on a ligand is

typically based on prior knowledge of structure-activity relationships of the ligand and/or congeners and/or structural information about ligand-receptor complexes (e.g., X-ray crystallography, NMR, and the like). Such positions and synthetic protocols for linkage are well known in the art and can be determined by those with ordinary skill in the art (*see, e.g.,*

5     **METHODS OF PREPARATION**, Examples 1-29 and Figures 7 to 21. Following attachment of a ligand to the linker or linkers, or to a significant portion thereof (e.g., 2-10 atoms of linker), the linker-ligand conjugate may be tested for retention of activity in a relevant assay system (*see Utility and Testing* below for representative assays).

10           At present, it is preferred that the multibinding compound is a bivalent compound in which two ligands are covalently linked, or a trivalent compound, in which three ligands are covalently linked. Linker design is further discussed under **METHODS OF PREPARATION**.

15           “Potency” as used herein refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its receptor. In some cases, the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multibinding agent and the aggregate of its unlinked ligand, the dose-response curve of each  
20     is determined under identical test conditions (e.g., in an *in vitro* or *in vivo* assay, in an appropriate animal model (such as a human patient)). The finding that the multibinding agent produces an equivalent biologic or therapeutic effect at a lower concentration than the aggregate unlinked ligand (e.g., on a per weight, per mole or per ligand basis) is indicative of enhanced potency.

25           “Selectivity” or “specificity” is a measure of the binding preferences of a ligand for different receptors. The selectivity of a ligand with respect to its target receptor relative to another receptor is given by the ratio of the respective values of  $K_d$  (i.e., the dissociation constants for each ligand-receptor complex) or, in cases where a biological effect is observed

below the  $K_d$ , the ratio of the respective  $EC_{50}$ s or  $IC_{50}$ s (i.e., the concentrations that produce 50% of the maximum response for the ligand interacting with the two distinct receptors).

The term "treatment" refers to any treatment of a disease or condition in a mammal, particularly a human, and includes:

- (i) preventing the disease or condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the condition and, accordingly, the treatment constitutes prophylactic treatment for the pathologic condition;
- (ii) inhibiting the disease or condition, i.e., arresting its development;
- (iii) relieving the disease or condition, i.e., causing regression of the disease or condition; or
- (iv) relieving the symptoms resulting from the disease or condition without addressing the underlying disease or condition, e.g., relieving symptoms of angina pectoris and other conditions of ischemia but not an underlying cause such as, for example, atherosclerotic disease or hypertension.

The phrase "disease or condition which is modulated by treatment with a multibinding  $K^+$  channel ligand" covers all disease states and/or conditions that are generally acknowledged in the art to be usefully treated with a ligand for a  $K^+$  channel in general, and those disease states and/or conditions that have been found to be usefully treated by a specific multibinding compound of our invention, i.e., the compounds of Formula I. Such disease states include, by way of example only, hypertension, cerebral ischemia, cardiac arrhythmias (particularly, arrhythmias resulting from potassium-related changes in membrane potential and conduction), cardiac hypertrophy due to systolic or diastolic overload, congestive heart failure, and the like.

The term "therapeutically effective amount" refers to that amount of multibinding compound that is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending

upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

5           The term "pharmaceutically acceptable excipient" is intended to include vehicles and carriers capable of being coadministered with a multibinding compound to facilitate the performance of its intended function. The use of such media for pharmaceutically active substances is well known in the art. Examples of such vehicles and carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. Any other conventional  
10 carrier suitable for use with the multibinding compounds also falls within the scope of the present invention.

## METHODS OF PREPARATION

### 15    Linkers

          The linker or linkers, when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multibinding compound. The biological activity of the multibinding K<sup>+</sup> channel compound is highly sensitive to the geometry, composition, size, length, flexibility or rigidity, the presence or absence of anionic  
20 or cationic charge, the relative hydrophobicity/hydrophilicity, and similar properties of the linker. Accordingly, the linker is preferably chosen to maximize the biological activity of the compound. The linker may be biologically "neutral," i.e., not itself contribute any additional biological activity to the multibinding compound, or it may be chosen to further enhance the biological activity of the compound. In general, the linker may be chosen from any organic  
25 molecule construct that orients two or more ligands for binding to the receptors to permit multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand-orienting result, and thus produce a multibinding compound.



For example, different orientations of ligands can be achieved by varying the geometry of the framework (linker) by use of mono- or polycyclic groups, such as aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). The optimal geometry and composition of frameworks (linkers) used in the multibinding compounds of this invention are based upon the properties of their intended receptors. For example, it is preferred to use rigid cyclic groups (e.g., aryl, heteroaryl), or non-rigid cyclic groups (e.g., cycloalkyl or crown groups) to reduce conformational entropy when such may be necessary to achieve energetically coupled binding.

Different hydrophobic/hydrophilic characteristics of the linker as well as the presence or absence of charged moieties can readily be controlled by the skilled artisan. For example, the hydrophobic nature of a linker derived from hexamethylene diamine ( $\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2$ ) or related polyamines can be modified to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group such as found in the commercially available "Jeffamines" (class of surfactants).

Different frameworks can be designed to provide preferred orientations of the ligands. The identification of an appropriate framework geometry for ligand domain presentation is an important first step in the construction of a multi binding agent with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process. Figure 2 illustrates a useful strategy for determining an optimal framework display orientation for ligand domains and can be used for preparing the bivalent compounds of this invention. Various alternative strategies known to those skilled in the art of molecular design can be substituted for the one described here.

As shown in Figure 2, the ligands (shown as filled circles) are attached to a central core structure such as phenyldiacetylene (Panel A) or cyclohexane dicarboxylic acid (Panel B). The ligands are spaced apart from the core by an attaching moiety of variable lengths  $m$

and  $n$ . If the ligand possesses multiple attachment sites (see discussion below), the orientation of the ligand on the attaching moiety may be varied as well. The positions of the display vectors around the central core structures are varied, thereby generating a collection of compounds. Assay of each of the individual compounds of a collection generated as  
5 described will lead to a subset of compounds with the desired enhanced activities (e.g., potency, selectivity). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will suggest a framework orientation that favors the properties desired.

The process may require the use of multiple copies of the same central core structure  
10 or combinations of different types of display cores. It is to be noted that core structures other than those shown here can be used for determining the optimal framework display orientation of the ligands. The above-described technique can be extended to trivalent compounds and compounds of higher-order valency.

A wide variety of linkers is commercially available (Chem Sources USA and Chem  
15 Sources International; the ACD electronic database; and Chemical Abstracts). Many of the linkers that are suitable for use in this invention fall into this category. Others can be readily synthesized by methods known in the art, and as described below. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like.  
20 Specific examples are peptides or polyamides, hydrocarbons, aromatics, heterocyclics, ethers, lipids, cationic or anionic groups, or a combination thereof.

Examples are given below and in Figure 3, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true  
25 spirit and scope of the invention. For example, properties of the linker can be modified by the addition or insertion of ancillary groups into the linker, for example, to change the solubility of the multibinding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto the linker enhances the

hydrophilicity and water solubility of the multibinding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further, PEG may decrease antigenicity and potentially enhances the overall rigidity of the linker.

5

Ancillary groups that enhance the water solubility/hydrophilicity of the linker, and accordingly, the resulting multibinding compounds, are useful in practicing this invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, small repeating units of ethylene glycols, alcohols, polyols, (e.g., glycerin, glycerol propoxylate, saccharides, including mono-, oligosaccharides, etc.) carboxylates (e.g., small repeating units of glutamic acid, acrylic acid, etc.), amines (e.g., tetraethylenepentamine), and the like to enhance the water solubility and/or hydrophilicity of the multibinding compounds of this invention. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyether. In particularly preferred embodiments, the ancillary group will contain a small number of repeating ethylene oxide ( $-\text{CH}_2\text{CH}_2\text{O}-$ ) units.

10  
15

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the compounds of Formula I is also within the scope of this invention. Lipophilic groups useful with the linkers of this invention include, but are not limited to, lower alkyl, aromatic groups and polycyclic aromatic groups. The aromatic groups may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. As used herein the term "aromatic groups" incorporates both aromatic hydrocarbons and heterocyclic aromatics. Other lipophilic groups useful with the linkers of this invention include fatty acid derivatives which may or may not form micelles in aqueous medium and other specific lipophilic groups which modulate interactions between the multibinding compound and biological membranes.

20

25

Also within the scope of this invention is the use of ancillary groups which result in the compound of Formula I being incorporated into a vesicle, such as a liposome, or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer or micelle such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidyl-choline, distearoyl-phosphatidylcholine and dilinoleoylphosphatidylcholine. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families, are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the linker can be manipulated by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker, or bonds between the linker and the ancillary group(s), or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational freedom is restrained by the presence of rings and/or  $\pi$ -bonds, for example, aryl, heteroaryl and heterocyclic groups. Other groups which can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the linker

into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other, which is inversely related to the square of the distance between the groups, will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups.

5 Further, ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker in a conformation which allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, protected groups that bear a latent charge which is  
10 unmasked, following addition to the linker, by deprotection, a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art, is within the scope of this invention.

Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal  
15 ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon  $\pi$ -bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

20

In preferred embodiments, rigidity (entropic control) is imparted by the presence of alicyclic (e.g., cycloalkyl), aromatic and heterocyclic groups. In other preferred  
embodiments, this comprises one or more six-membered rings. In still further preferred  
embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl, or a  
25 macrocyclic ring such as, for example, a crown compound.

In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art.

Eliminating or reducing antigenicity of the multibinding compounds described herein is also within the scope of this invention. In certain cases, the antigenicity of a multibinding compound may be eliminated or reduced by use of groups such as, for example, poly(ethylene glycol).

5

#### The Compounds of Formula I

As explained above, the multibinding compounds described herein comprise 2-10 ligands attached covalently to a linker that links the ligands in a manner that allows their multivalent binding to ligand binding sites of  $K^+$  channels. The linker spatially constrains these interactions to occur within dimensions defined by the linker. This and other factors increases the biologic and/or therapeutic effect of the multibinding compound as compared to the same number of ligands used in monobinding form.

10

The compounds of this invention are preferably represented by the empirical formula  $(L)_p(X)_q$  where L, X, p and q are as defined above. This is intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is provided below.

15

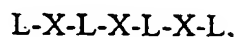
As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain or at any intermediate position thereof.

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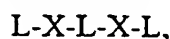
The simplest and most preferred multibinding compound is a bivalent compound which can be represented as L-X-L, where L is a ligand and is the same or different and X is the linker. A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as is X. However, a trivalent compound can also comprise three ligands attached to a central core, and thus be represented as  $(L)_3X$ , where the linker X could

25

include, for example, an aryl or cycloalkyl group. Tetravalent compounds can be represented in a linear array:



5 or a branched array:



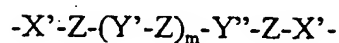
10 i.e., a branched construct analogous to the isomers of butane (*n*-butyl, *iso*-butyl, *sec*-butyl, and *t*-butyl). Alternatively, it could be represented as an aryl or cycloalkyl derivative as described above with four (4) ligands attached to the core linker.

15 The same considerations apply to higher multibinding compounds of this invention containing from 5-10 ligands. However, for multibinding agents attached to a central linker such as an aryl, cycloalkyl or heterocyclyl group, or a crown compound, there is a self-evident constraint that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of  
20 ligands.

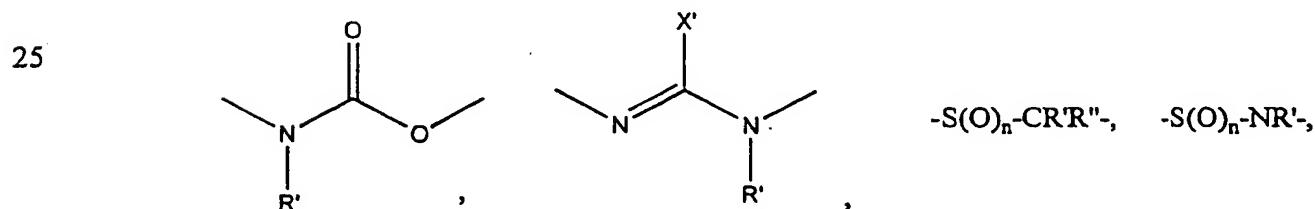
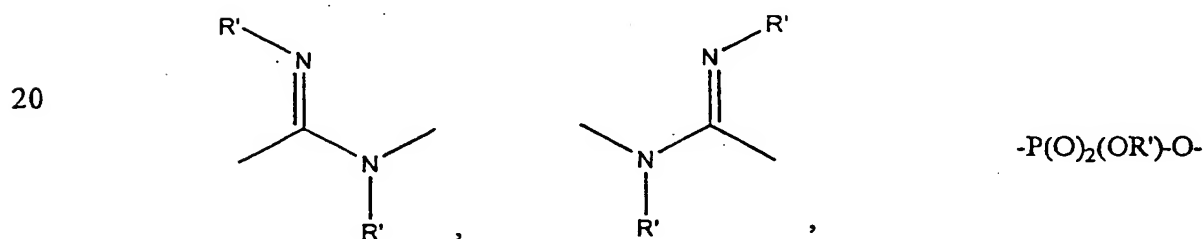
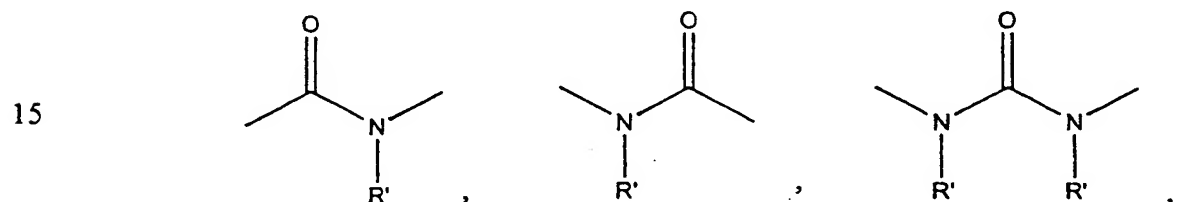
The formula  $(\text{L})_p(\text{X})_q$  is also intended to represent a cyclic compound of formula  $(-\text{L}-\text{X}-)_n$ , where *n* is 2-10.

25 All of the above variations are intended to be within the scope of the invention defined by the formula  $(\text{L})_p(\text{X})_q$ . Examples of bivalent and higher-order valency compounds of this invention are provided in Figures 4A to 4D.

With the foregoing in mind, a preferred linker may be represented by the following formula:



- 5 in which:  $m$  is an integer of from 0 to 20;  $X'$  at each separate occurrence is  $-O-$ ,  $-S-$ ,  $-S(O)-$ ,  $-S(O)_2-$ ,  $-NR-$ ,  $-N^+R R'-$ ,  $-C(O)-$ ,  $-C(O)O-$ ,  $-C(O)NH-$ ,  $-C(S)-$ ,  $-C(S)O-$ ,  $-C(S)NH-$  or a covalent bond, where  $R$  and  $R'$  at each separate occurrence are as defined below for  $R'$  and  $R''$ ;  $Z$  is at each separate occurrence selected from alkylene, substituted alkylene, alkylalkoxy, cycloalkylene, substituted cycloalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted alkenylene, arylene, substituted arylene, heteroarylene, heterocyclene, substituted heterocyclene, crown compounds, or a covalent bond;  $Y'$  and  $Y''$  at each separate occurrence are selected from the group consisting of





-S-S- or a covalent bond; in which:  $n$  is 0, 1 or 2; and  $R'$  and  $R''$  at each separate occurrence are selected from hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl or heterocyclic.

5            Additionally, the linker moiety can be optionally substituted at any atom therein by one or more alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic group.

10            As indicated above, the simplest (and preferred) construct is a bivalent compound which can be represented as  $L-X-L$ , where  $L$  is a  $K^+$  channel ligand that is the same or different at each occurrence, and  $X$  is the linker. Accordingly, examples of the preparation of a bivalent ligand are given below as an illustration of the manner in which multibinding compounds of Formula I are obtained.

15            The reaction schemes that follow illustrate preferred linking strategies for linking phenylmethane sulfonamide (dofetilide, ibutilide, sotalol, and E-4031) and benzofuran (amiodarone, desethylamiodarone, NE-10064) classes of potassium channel modulators. These strategies are intended to apply as well to any  $K^+$  channel ligand that includes, or can be functionalized with groups compatible with the chosen linker (e.g.,  
20            azimilide and tedisamil).

              As was previously discussed, the linker or linkers can be attached to different positions on the ligand molecule to achieve different orientations of the ligand domains and thereby facilitate multivalency. For example, the positions that are potentially available for  
25            linking a benzofuran such as amiodarone are indicated by arrows in the structure shown in Figure 5.

              Preferred positions of attachment suggested by known SAR are illustrated in the reaction schemes of Figures 7 to 21. Examples of ligands are shown in Table 4.

Certain  $K^+$  channel ligands may be chiral and exhibit stereoselectivity. The most active enantiomers are preferably used as ligands in the multibinding compounds of this invention. The chiral resolution of enantiomers is accomplished by well known procedures that result in the formation of diastereomeric derivatives or salts, followed by conventional separation by chromatographic procedures or by fractional crystallization (*see, e.g.*, Bossert, et al., *Angew. Chem. Int. Ed.*, 20:762-769 (1981) and U.S. Patent No. 5,571,827 and references cited therein). They may also be obtained by asymmetric synthesis.

The ligands are covalently attached to the linker using conventional chemical techniques. The reaction chemistries resulting in such linkage are well known in the art and involve the coupling of reactive functional groups present on the linker and ligand. In some cases, it may be necessary to protect portions of the ligand that are not involved in linking reactions. Protecting groups for this purpose are well known in the art and are indicated generally in the reaction schemes by the symbols PG and PG'.

Preferably, the reactive functional groups on the linker are selected relative to the functional groups on the ligand that are available for coupling, or can be introduced onto the ligand for this purpose. In some embodiments, the linker is coupled to ligand precursors, with the completion of ligand synthesis being carried out in a subsequent step. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as J. March, *Advanced Organic Chemistry*, 4<sup>th</sup> Ed. (Wiley- Interscience, N.Y., 1992). Examples of the chemistry for connecting ligands by a linker are shown in Figure 6, where  $R^1$  and  $R^2$  represent a ligand and/or the linking group. One skilled in the art will appreciate that synthetically equivalent coupling reactions can be substituted for the reactions illustrated herein.

The linker to which the ligands or ligand precursors are attached comprises a "core" molecule having two or more functional groups with reactivity that is complementary to that of the functional groups on the ligand. Figure 3 illustrates the diversity of "cores" that are

useful for varying the linker size, shape, length, orientation, rigidity, acidity/basicity, hydrophobicity/hydrophilicity, hydrogen bonding characteristics and number of ligands connected. This pictorial representation is intended only to illustrate the invention, and not to limit its scope to the structures shown. In the Figures and reaction schemes that follow, a solid circle is used to generically represent a core molecule, referred to as "Link" in the Examples. The solid circle is equivalent to a linker as defined above after reaction.

The preferred compounds of Formula I are bivalent. Accordingly, and for the purpose of simplicity, most of the figures and reaction schemes below illustrate the synthesis of bivalent  $K^+$  channel modulators. It should be noted, however, that the same techniques can be used to generate higher order multibinding compounds, i.e., the compounds of the invention where p is 3-10. (See, e.g., Figure 15 and 20.)

Reactions performed under standard amide coupling conditions are carried out in an inert polar solvent (e.g., DMF, DMA) in the presence of a hindered base (e.g., TEA, DIPEA) and standard amide coupling reagents (e.g., DPPA, PyBOP, HATU, DCC).

Several methods for preparing bivalent benzofuran (BF) compounds, as exemplified here for amiodarone and structurally analogous molecules, are illustrated in the reaction schemes for amiodarone and dronedarone shown in Figure 7. These are described in detail in Examples 1-3.

Several methods for preparing bivalent phenylmethane sulfonamide (PMS) compounds, as exemplified by dofetilide, ibutilide, sematilide and sotalol, and structurally analogous molecules are illustrated in the reaction schemes shown in Figures 8 - 11. These are described in detail in Examples 4-11.

Several methods for preparing bivalent azimilide and tedisamil compounds are illustrated in the reaction schemes shown in Figures 12 - 13. These are described in detail in Examples 12 - 14.

5           The strategies for preparing compounds of Formula I discussed above involve coupling the ligand directly to a homobifunctional core. Another strategy that can be used with all ligands, and for the preparation of both bivalent and higher order multibinding compounds, is to introduce a 'spacer' before coupling to a central core. Such a spacer can itself be selected from the same set as the possible core compounds. Examples of this  
10       linking strategy using starting materials prepared as described above, are shown in Figure 14, where the spacer is represented by a black circle. As defined herein, the linker comprises the spacer + core. These are described in detail in Examples 15-17.

15           Compounds of Formula I of higher order valency, i.e.,  $p > 2$ , can be prepared by simple extension of the above strategies. As shown in Figure 15, compounds are prepared by coupling ligands to a central core bearing multiple functional groups. The reaction conditions are the same as described above for the preparation of bivalent compounds, with appropriate adjustments made in the molar quantities of ligand and reagents. These are described in detail in Examples 18-21.

20           Figures 16 and 17 show ligands coupled to a polypeptide core with a sidechain spacer. Solid phase peptide synthesis can be used to produce a wide variety of peptidic core molecules. Techniques well-known to those skilled in the art (including combinatorial methods) are used to vary the distance between ligand attachment sites on the core molecule,  
25       the number of attachment sites available for coupling, and the chemical properties of the core molecule. Orthogonal protecting groups are used to selectively protect functional groups on the core molecule, thus allowing ancillary groups to be inserted into the linker of the multibinding compound and/or the preparation of "heterovalomers" (i.e., multibinding compounds with nonidentical ligands).

All of the synthetic strategies described above employ a step in which the ligand, attached to spacers or not, is symmetrically linked to functionally equivalent positions on a central core. Compounds of Formula I can also be synthesized using an asymmetric linear approach. This strategy is preferred when linking two or more ligands at different points of connectivity (*see, e.g.*, Figure 18) or when preparing heterovalomers (*see, e.g.*, Figure 19). These are described in detail in Examples 22-25.

#### Isolation and Purification of the Compounds

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. Characterization is preferably by NMR and mass spectroscopy.

#### Utility and Testing

The multibinding compounds of this invention can be used to modulate potassium channels in various tissues including heart, muscle, and neurons. They will typically be used for the treatment of diseases and conditions in mammals that involve or are mediated by K<sup>+</sup> channels, such as hypertension, cardiac arrhythmias, cerebral ischemia, congestive heart failure, and the like.

The multibinding compounds of this invention are tested in well-known and reliable assays and their activities are compared with those of the corresponding unlinked (i.e., monovalent) ligands.

#### Binding affinity to potassium channels

The binding affinity is determined by a radioligand competitive inhibition assay.<sup>23</sup> The ability of the present compounds to compete with [<sup>3</sup>H]dofetilide or a similar radioactive ligand in binding to high- and low-affinity binding sites of guinea pig ventricular myocytes is

measured *in vitro*. The binding affinity, calculated from competition curves, is compared with that of the monovalent ligand and/or monovalent linker-ligand conjugate.

#### Antiarrhythmic effect

5           Antiarrhythmic effect of compounds of this invention may be determined *in vivo* in dogs with induced myocardial infarction and reproducibly inducible ventricular tachycardia or ventricular fibrillation.<sup>1,22</sup> Suppression of inducible arrhythmias is measured.

10           The antifibrillatory and antiarrhythmic effects of the compounds of this invention may be determined *in vivo* using a canine model of sudden death.<sup>7</sup> Reduction of the incidence of programmed electrical stimulation (PES) induced ventricular tachycardia and protection against ischemia-induced ventricular fibrillation are measured.

15           The antiarrhythmic effect of the compounds of this invention may be determined *in vivo* using the mouse chloroform model.<sup>8</sup> The percentage of animals showing normal sinus rhythm is measured.

20           The antiarrhythmic effect of the compounds of this invention may be determined *in vivo* using the rat coronary ligation model.<sup>8</sup> Ventricular extrasystoles occurring during the 30 minutes following the procedure are counted.

25           The antiarrhythmic effect of the compounds of this invention may be determined *in vitro* or *in vivo* using rat coronary artery ligation/reperfusion models.<sup>8</sup> In the *in vitro* model, excised rat hearts are retrogradely perfused with a solution of the compound to be tested, then the coronary artery is ligated, followed by reperfusion. In the *in vivo* evaluation, the compound is administered i.p., then the coronary artery is ligated, followed by reperfusion. In both models the incidence and time to onset during reperfusion of ventricular extrasystole, tachyarrhythmia and fibrillation are measured.

The antiarrhythmic effect of the compounds of this invention may be determined *in vivo* using an anesthetized rat model of ventricular arrhythmias.<sup>9</sup> The time to onset of ventricular extrasystoles is measured.

5           The antiarrhythmic effect of the compounds of this invention may be determined *in vivo* using a canine myocardial infarction model where compound is administered 24 hours after ligation of the left anterior descending coronary artery.<sup>11</sup> Right ventricular effective refractory period, monophasic action potential duration and reduction of PES induced ventricular tachycardia and ventricular fibrillation are measured.

10

The ability of the compounds of this invention to prolong the action potential (achieve a slower onset of active state block) and recover faster from block may be determined *in vitro* using rabbit ventricular myocytes.<sup>13,30</sup> Development of block during a long depolarizing clamp and recovery from block are measured.

15

The ability of the compounds of this invention to suppress repolarization arrhythmias may be determined *in vitro* using canine epicardium midmyocardium and endocardium and canine cardiac Purkinje fibers and *in vivo* using anesthetized rabbits.<sup>26,58,62</sup>

20

The ability of the compounds of this invention to suppress arrhythmias may be determined *in vivo* using the feline coronary occlusion and left stellate ganglion stimulation model, the conscious canine model of transient ischemia during exercise in the presence of a healed MI and the conscious canine model of complete occlusion after recent MI.<sup>52,59</sup>

25

The ability of the compounds of this invention to prolong action potential duration may be determined *in vivo* and *in vitro* using guinea pig hearts,<sup>57</sup> and *in vitro* using calf cardiac Purkinje fibers.<sup>63</sup>

The ability of the compounds of this invention to prevent atrial fibrillation (AF) may be determined using a canine model of sustained vagotonic AF.<sup>68</sup> Prevention of AF induction is measured. Reverse use dependence may also be determined.

5     Effect on tachycardia

The effect of compounds of this invention on tachycardia may be determined *in vitro* using rabbit right atrial preparations.<sup>2</sup> Micro-electrode techniques are used to measure the ability to prolong the refractory period and thus prevent initiation of tachycardia.

10     The effect of the compounds of this invention on tachyarrhythmias may be determined *in vitro* using guinea pig right ventricular papillary muscle.<sup>27</sup> The action potential duration at different extracellular potassium concentrations is measured.

Effect on potassium currents

15     The effect of compounds of this invention on the repolarization currents  $I_K$  and  $I_{To}$  may be determined *in vitro* using whole cell recordings in cat ventricular myocytes and papillary muscles from the hearts of oophorectomized rabbits.<sup>4,31</sup>

20     The effect of compounds of this invention on various specific potassium current may be determined *in vitro* using guinea pig ventricular myocytes and sinoatrial node cells, human atrial myocytes, canine ventricular muscle and Purkinje fibers, guinea pig papillary muscle, single voltage clamped guinea pig ventricular myocytes and human ventricular endomyocardium.<sup>6,12,17,25,29,32,33,37,51,66,69,71</sup>

25     The ability of compounds of this invention to inhibit potassium currents in a non-cardiac preparation may be determined using rat taste receptor cells.<sup>60</sup>



Selectivity and/or Specificity:

The ability of compounds of this invention to modulate the KATP channel may be determined using a  $^{86}\text{Rb}$  efflux assay.<sup>15,64</sup> Thus, this is a potency assay.

5           The selectivity and/or specificity of the compounds of this invention may be determined using CHO cell lines expressing specific recombinant potassium channel subtypes.<sup>34</sup>

10           The selectivity of compounds of this invention for various potassium channel currents may be determined *in vitro* using cloned K channels expressed in cells or ventricular myocytes.<sup>12,34</sup>

15           The selectivity of compounds of this invention for various receptors may be determined *in vitro* using rat synaptosomal membrane. (Pong, et al. "Binding profile of NE-10064, a novel Class III anti-arrhythmic agent to rat brain receptors", *Faseb J.*, 7:A474 (1993)).

Antivasoconstrictor activity

20           Antivasoconstrictor activity is determined as described in Brittain, et al., *Physiologist*, 28:325 (1985) as the concentration of a compound required to produce 50% vasorelaxation in KCl-contracted rabbit thoracic aorta strips in the presence of calcium. Alternatively, the concentration of a compound required to inhibit coronary vasoconstriction induced by a thromboxane mimetic (U-46619, i.e., 9,11-methanoepoxy-PGH<sub>2</sub>) in guinea pig Langendorff heart preparation is measured as described in Eltze, et al., *Chirality*, 2:233-240 (1990).

25

Antihypertensive activity

          Antihypertensive activity is determined in male spontaneously hypertensive rats by measurement of mean arterial blood pressure (Rovnyak, et al., *J. Med. Chem.*, 35:3254-3263 (1992)).

### Tissue selectivity

Selectivity for vascular smooth muscle as compared with cardiac muscle can be assessed by comparing the concentration of a multibinding compound that produces a 50% increase in coronary blood flow in an isolated guinea-pig heart with that required to inhibit myocardial contractility. See, e.g., Osterrieder, W. and Holck, M., *J. Cardiovasc. Pharm.*, 13:754-9 (1989); and Cremers, et al., *J. Cardiovasc. Pharm.*, 29:692-696 (1997).

### Combinatorial Libraries

The methods described above lend themselves to combinatorial approaches for identifying multimeric compounds which possess multibinding properties for potassium channels.

Specifically, factors such as the proper juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding sites on a target or targets is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for a particular target. These parameters include: (1) the identity of ligand(s), (2) the orientation of ligands, (3) the valency of the construct, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:

### Selection of ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed against a particular biological target or targets. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s). Thus, ligands may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short *in vivo* half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

### Orientation: selection of ligand attachment points and linking chemistry

Several points are chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting

the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a protease inhibitor bound to its target allows one to identify one or more sites where linker attachment will not preclude the enzyme:inhibitor interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a receptor antagonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the formal antagonist binding

site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in monomeric form.

5           The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands that bind to common or different targets. For  
10       example, a 5HT<sub>4</sub> receptor antagonist and a bladder-selective muscarinic M<sub>3</sub> antagonist may be joined to a linker through attachment points which do not abrogate the binding affinity of the monomeric ligands for their respective receptor sites. The dimeric compound may achieve enhanced affinity for both receptors due to favorable interactions between the 5HT<sub>4</sub> ligand and elements of the M<sub>3</sub> receptor proximal to the formal M<sub>3</sub> antagonist binding site  
15       and between the M<sub>3</sub> ligand and elements of the 5HT<sub>4</sub> receptor proximal to the formal 5HT<sub>4</sub> antagonist binding site. Thus, the dimeric compound may be more potent and selective antagonist of overactive bladder and a superior therapy for urinary urge incontinence.

20           Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically innocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are  
25       but a few examples of preferred linkages.

Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into  
5 consideration the following factors:

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than  
10 sufficient to confer biological advantage. Furthermore, divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the spanning of a  
15 range of inter-ligand distances that encompass the distance preferable for a given divalent interaction.. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets, typically enzymes and soluble receptor targets. In other instances where high-resolution structural information is not available  
20 (such as 7TM G-protein coupled receptors), one can make use of simple models to estimate the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are 2-20 Å, with more preferred linker distances of 3-12 Å. In situations where two binding sites  
25 reside on separate (e.g., protein) target sites, preferred linker distances are 20-100 Å, with more preferred distances of 30-70 Å.

Linker geometry and rigidity. The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in which the

ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two ligands to the ortho, meta, and para positions of a benzene ring, or in *cis*- or *trans*-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around a cyclohexane core or in *cis*- or *trans*-arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

Linker physical properties. The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarization, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

### Combinatorial synthesis

Having chosen a set of  $n$  ligands ( $n$  being determined by the sum of the number of different attachment points for each ligand chosen) and  $m$  linkers by the process outlined above, a library of  $(n!)m$  candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalities on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or linker is attached to a solid support. Alternatively and preferably, the combinatorial library is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

### Analysis of array by biochemical, analytical, pharmacological, and computational methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess



multibinding properties. Physical constants such as solubility under various solvent conditions and logD/clogD values can be determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition analysis for many enzyme targets. *In vitro* efficacy, such as for receptor agonists and antagonists, ion channel blockers, and antimicrobial activity, can also be determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data can be determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

The members of the library which exhibit multibinding properties, as defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both *in vitro* and *in vivo*).

Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libraries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No.

2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative binding affinities of candidate multibinding compounds to receptors.

- 5           The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

Follow-up synthesis and analysis of additional array(s)

- 10           Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries can then be generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure in an effort to further optimize target
- 15           affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

20

- To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates, diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case,
- 25           the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

## COMPLEMENTARY BINDING CHEMISTRIES

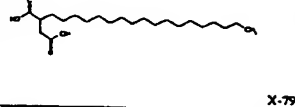
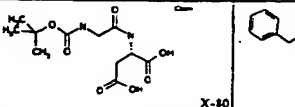
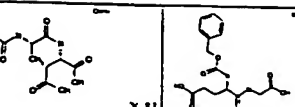
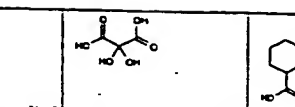

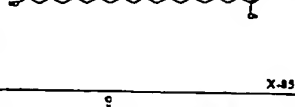
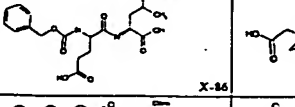
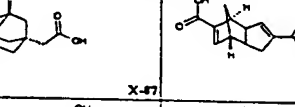
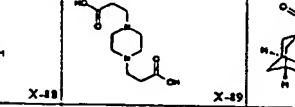
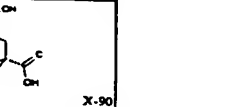
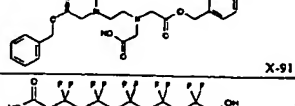
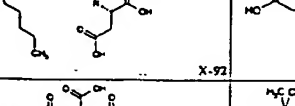
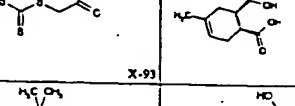
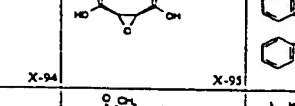
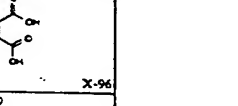
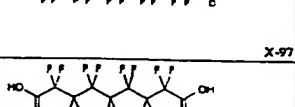
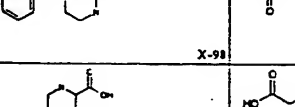

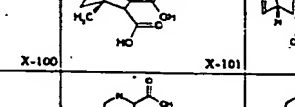
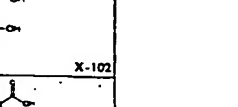
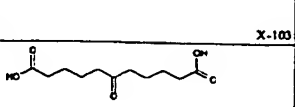
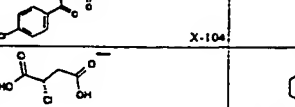
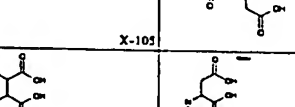
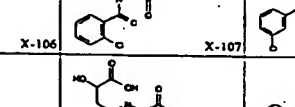
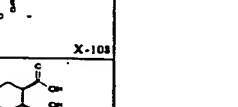
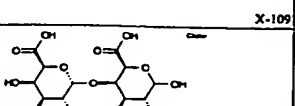
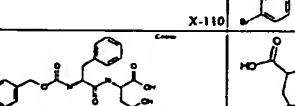
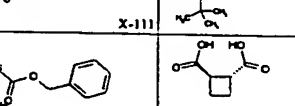
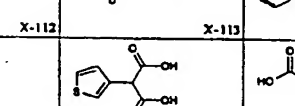
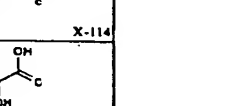
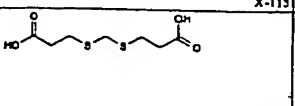
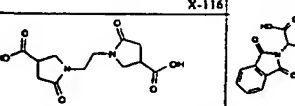
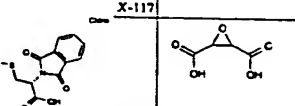
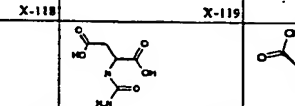
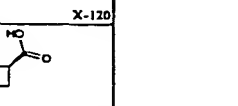
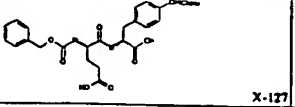
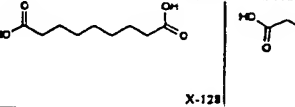
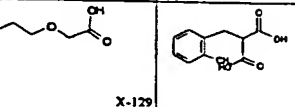
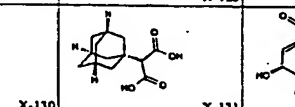
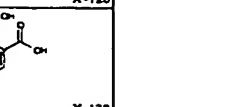
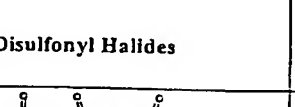

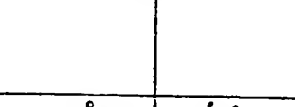

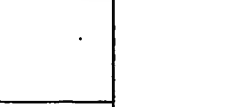
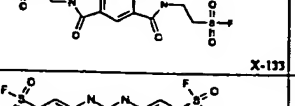
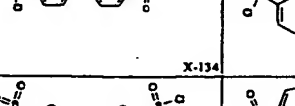
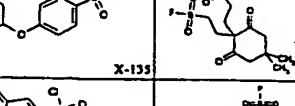
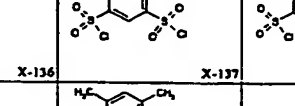
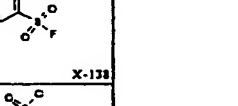
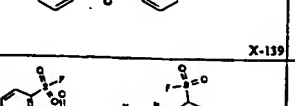
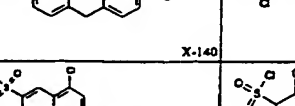
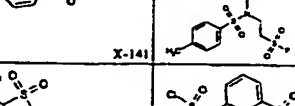
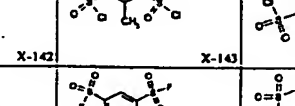
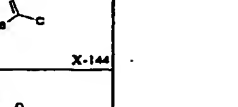
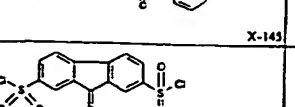
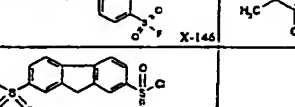
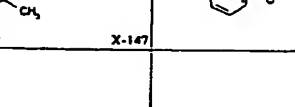
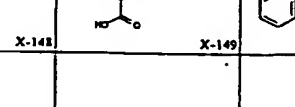
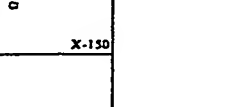
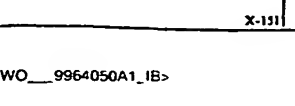
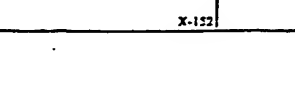
	<u>First Reactive Group</u>	<u>Second Reactive Group</u>	<u>Linkage</u>
	hydroxyl	isocyanate	urethane
5	amine	epoxide	$\beta$ -hydroxyamine
	sulfonyl halide	amine	sulfonamide
	carboxyl acid	amine	amide
	hydroxyl	alkyl/aryl halide	ether
	aldehyde	amine/ $\text{NaCNBH}_4$	amine
10	ketone	amine/ $\text{NaCNBH}_4$	amine
	amine	isocyanate	carbamate

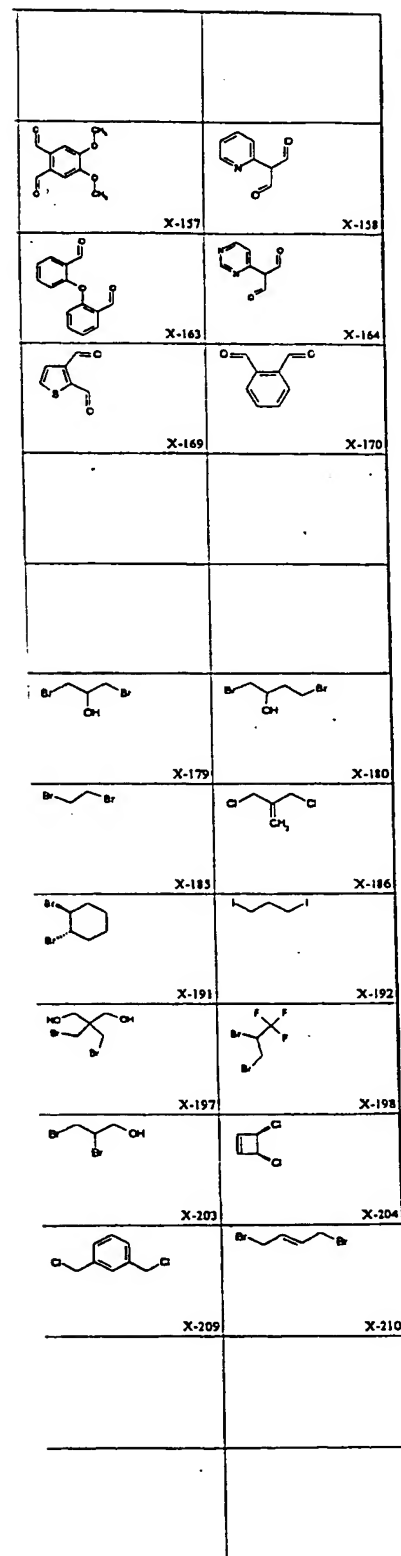
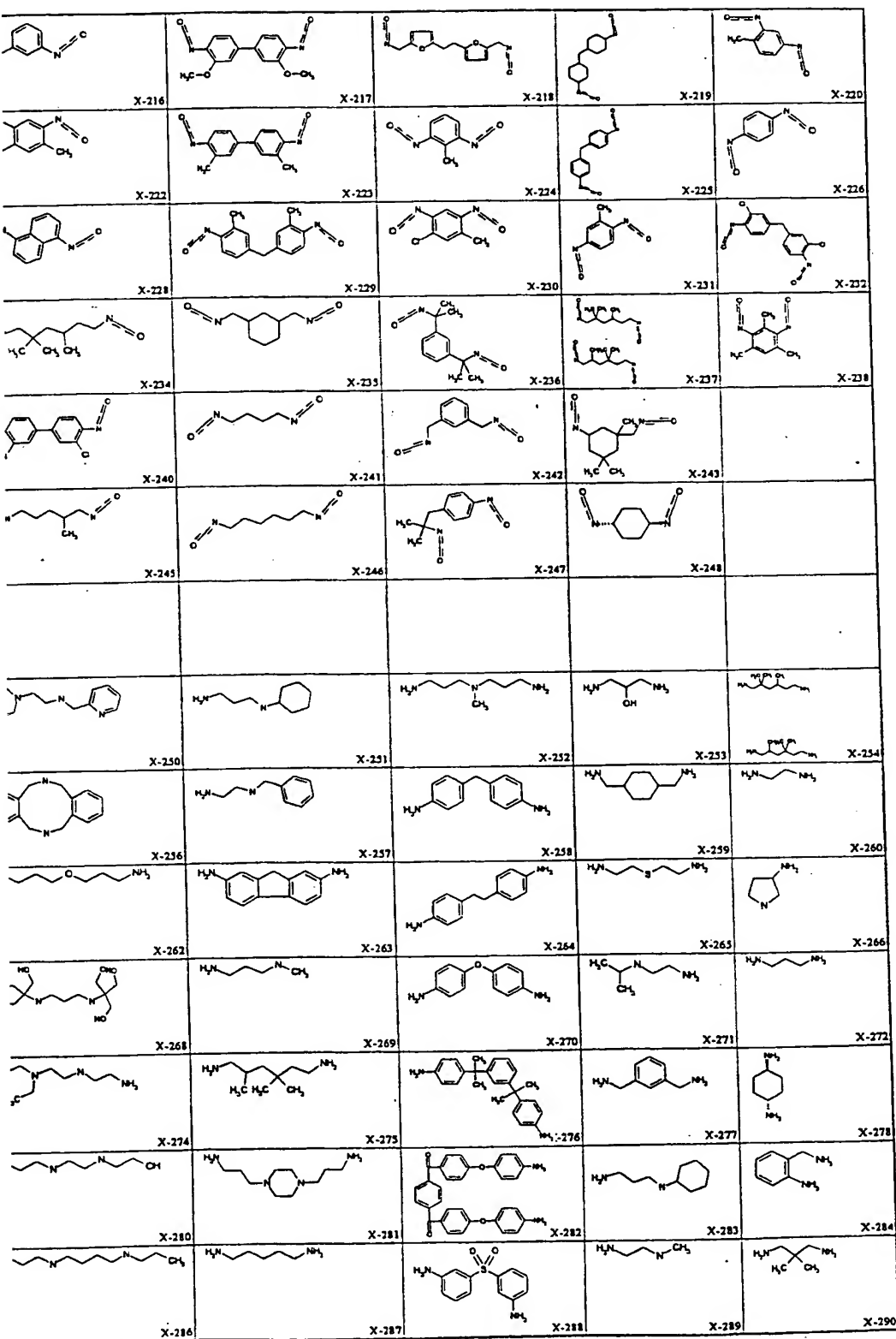
Exemplary linkers include the following linkers identified as X-1 through X-418 as set forth below:

15

20

<b>Diacids</b>					
X-1	X-2	X-3	X-4	X-5	X-6
X-7	X-8	X-9	X-10	X-11	X-12
X-13	X-14	X-15	X-16	X-17	X-18
X-19	X-20	X-21	X-22	X-23	X-24
X-25	X-26	X-27	X-28	X-29	X-30
X-31	X-32	X-33	X-34	X-35	X-36
X-37	X-38	X-39	X-40	X-41	X-42
X-43	X-44	X-45	X-46	X-47	X-48
X-49	X-50	X-51	X-52	X-53	X-54
X-55	X-56	X-57	X-58	X-59	X-60
X-61	X-62	X-63	X-64	X-65	X-66
X-67	X-68	X-69	X-70	X-71	X-72
X-73	X-74	X-75	X-76	X-77	X-78

					
					
					
					
					
					
					
					
					
<b>Disulfonyl Halides</b>					
					
					
					
					



X-368	X-369	X-370	X-371	X-372	X-373
X-374	X-375	X-376	X-377	X-378	X-379
X-380	X-381	X-382	X-383	X-384	X-385
<b>Dithiols</b>					
X-386	X-387	X-388	X-389	X-390	X-391
X-392	X-393	X-394	X-395	X-396	X-397
X-398	X-399	X-400	X-401	X-402	X-403
X-404	X-405	X-406	X-407	X-408	X-409
X-410	X-411	X-412	X-413	X-414	X-415
X-416	X-417	X-418			

Representative ligands for use in this invention include, by way of example, L-1 through L-4. L-1 ligands are benzofuran compounds (e.g., 7A-1, 7B-1 or 7C-1 of Examples 1-3). Phenylmethane sulfonamide structures are designated L-2 ligands (e.g., 8A-1, 8B-1, 8C-1, 9A-1, 9B-1, 10A-1, 10B-1, or 11-1 of Examples 4-11). L-3 ligands are azimilide compounds (e.g., 12-1, 12-3 of Examples 12-13). L-4 ligands are tedisamil compounds (e.g., 13-1 of Example 14).

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 through L-4 above and the second ligand and linker is selected from the following:

	L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
	L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
	L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
15	L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
	L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
	L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
	L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
	L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
20	L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
	L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
	L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
	L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
	L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
25	L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
	L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-
	L-1/X-91-	L-1/X-92-	L-1/X-93-	L-1/X-94-	L-1/X-95-	L-1/X-96-
	L-1/X-97-	L-1/X-98-	L-1/X-99-	L-1/X-100-	L-1/X-101-	L-1/X-102-



	L-1/X-103-	L-1/X-104-	L-1/X-105-	L-1/X-106-	L-1/X-107-	L-1/X-108-
	L-1/X-109-	L-1/X-110-	L-1/X-111-	L-1/X-112-	L-1/X-113-	L-1/X-114-
	L-1/X-115-	L-1/X-116-	L-1/X-117-	L-1/X-118-	L-1/X-119-	L-1/X-120-
	L-1/X-121-	L-1/X-122-	L-1/X-123-	L-1/X-124-	L-1/X-125-	L-1/X-126-
5	L-1/X-127-	L-1/X-128-	L-1/X-129-	L-1/X-130-	L-1/X-131-	L-1/X-132-
	L-1/X-133-	L-1/X-134-	L-1/X-135-	L-1/X-136-	L-1/X-137-	L-1/X-138-
	L-1/X-139-	L-1/X-140-	L-1/X-141-	L-1/X-142-	L-1/X-143-	L-1/X-144-
	L-1/X-145-	L-1/X-146-	L-1/X-147-	L-1/X-148-	L-1/X-149-	L-1/X-150-
	L-1/X-151-	L-1/X-152-	L-1/X-153-	L-1/X-154-	L-1/X-155-	L-1/X-156-
10	L-1/X-157-	L-1/X-158-	L-1/X-159-	L-1/X-160-	L-1/X-161-	L-1/X-162-
	L-1/X-163-	L-1/X-164-	L-1/X-165-	L-1/X-166-	L-1/X-167-	L-1/X-168-
	L-1/X-169-	L-1/X-170-	L-1/X-171-	L-1/X-172-	L-1/X-173-	L-1/X-174-
	L-1/X-175-	L-1/X-176-	L-1/X-177-	L-1/X-178-	L-1/X-179-	L-1/X-180-
	L-1/X-181-	L-1/X-182-	L-1/X-183-	L-1/X-184-	L-1/X-185-	L-1/X-186-
15	L-1/X-187-	L-1/X-188-	L-1/X-189-	L-1/X-190-	L-1/X-191-	L-1/X-192-
	L-1/X-193-	L-1/X-194-	L-1/X-195-	L-1/X-196-	L-1/X-197-	L-1/X-198-
	L-1/X-199-	L-1/X-200-	L-1/X-201-	L-1/X-202-	L-1/X-203-	L-1/X-204-
	L-1/X-205-	L-1/X-206-	L-1/X-207-	L-1/X-208-	L-1/X-209-	L-1/X-210-
	L-1/X-211-	L-1/X-212-	L-1/X-213-	L-1/X-214-	L-1/X-215-	L-1/X-216-
20	L-1/X-217-	L-1/X-218-	L-1/X-219-	L-1/X-220-	L-1/X-221-	L-1/X-222-
	L-1/X-223-	L-1/X-224-	L-1/X-225-	L-1/X-226-	L-1/X-227-	L-1/X-228-
	L-1/X-229-	L-1/X-230-	L-1/X-231-	L-1/X-232-	L-1/X-233-	L-1/X-234-
	L-1/X-235-	L-1/X-236-	L-1/X-237-	L-1/X-238-	L-1/X-239-	L-1/X-240-
	L-1/X-241-	L-1/X-242-	L-1/X-243-	L-1/X-244-	L-1/X-245-	L-1/X-246-
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	L-1/X-253-	L-1/X-254-	L-1/X-255-	L-1/X-256-	L-1/X-257-	L-1/X-258-
	L-1/X-259-	L-1/X-260-	L-1/X-261-	L-1/X-262-	L-1/X-263-	L-1/X-264-
	L-1/X-265-	L-1/X-266-	L-1/X-267-	L-1/X-268-	L-1/X-269-	L-1/X-270-

	L-1/X-271-	L-1/X-272-	L-1/X-273-	L-1/X-274-	L-1/X-275-	L-1/X-276-
	L-1/X-277-	L-1/X-278-	L-1/X-279-	L-1/X-280-	L-1/X-281-	L-1/X-282-
	L-1/X-283-	L-1/X-284-	L-1/X-285-	L-1/X-286-	L-1/X-287-	L-1/X-288-
	L-1/X-289-	L-1/X-290-	L-1/X-291-	L-1/X-292-	L-1/X-293-	L-1/X-294-
5	L-1/X-295-	L-1/X-296-	L-1/X-297-	L-1/X-298-	L-1/X-299-	L-1/X-300-
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	L-1/X-307-	L-1/X-308-	L-1/X-309-	L-1/X-310-	L-1/X-311-	L-1/X-312-
	L-1/X-313-	L-1/X-314-	L-1/X-315-	L-1/X-316-	L-1/X-317-	L-1/X-318-
	L-1/X-319-	L-1/X-320-	L-1/X-321-	L-1/X-322-	L-1/X-323-	L-1/X-324-
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	L-2/X-91-	L-2/X-92-	L-2/X-93-	L-2/X-94-	L-2/X-95-	L-2/X-96-
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	L-2/X-103-	L-2/X-104-	L-2/X-105-	L-2/X-106-	L-2/X-107-	L-2/X-108-
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25	L-2/X-157-	L-2/X-158-	L-2/X-159-	L-2/X-160-	L-2/X-161-	L-2/X-162-
	L-2/X-163-	L-2/X-164-	L-2/X-165-	L-2/X-166-	L-2/X-167-	L-2/X-168-
	L-2/X-169-	L-2/X-170-	L-2/X-171-	L-2/X-172-	L-2/X-173-	L-2/X-174-
	L-2/X-175-	L-2/X-176-	L-2/X-177-	L-2/X-178-	L-2/X-179-	L-2/X-180-

	L-2/X-181-	L-2/X-182-	L-2/X-183-	L-2/X-184-	L-2/X-185-	L-2/X-186-
	L-2/X-187-	L-2/X-188-	L-2/X-189-	L-2/X-190-	L-2/X-191-	L-2/X-192-
	L-2/X-193-	L-2/X-194-	L-2/X-195-	L-2/X-196-	L-2/X-197-	L-2/X-198-
	L-2/X-199-	L-2/X-200-	L-2/X-201-	L-2/X-202-	L-2/X-203-	L-2/X-204-
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	L-2/X-211-	L-2/X-212-	L-2/X-213-	L-2/X-214-	L-2/X-215-	L-2/X-216-
	L-2/X-217-	L-2/X-218-	L-2/X-219-	L-2/X-220-	L-2/X-221-	L-2/X-222-
	L-2/X-223-	L-2/X-224-	L-2/X-225-	L-2/X-226-	L-2/X-227-	L-2/X-228-
	L-2/X-229-	L-2/X-230-	L-2/X-231-	L-2/X-232-	L-2/X-233-	L-2/X-234-
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	L-2/X-241-	L-2/X-242-	L-2/X-243-	L-2/X-244-	L-2/X-245-	L-2/X-246-
	L-2/X-247-	L-2/X-248-	L-2/X-249-	L-2/X-250-	L-2/X-251-	L-2/X-252-
	L-2/X-253-	L-2/X-254-	L-2/X-255-	L-2/X-256-	L-2/X-257-	L-2/X-258-
	L-2/X-259-	L-2/X-260-	L-2/X-261-	L-2/X-262-	L-2/X-263-	L-2/X-264-
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	L-2/X-343-	L-2/X-344-	L-2/X-345-	L-2/X-346-	L-2/X-347-	L-2/X-348-

	L-2/X-349-	L-2/X-350-	L-2/X-351-	L-2/X-352-	L-2/X-353-	L-2/X-354-
	L-2/X-355-	L-2/X-356-	L-2/X-357-	L-2/X-358-	L-2/X-359-	L-2/X-360-
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	L-2/X-379-	L-2/X-380-	L-2/X-381-	L-2/X-382-	L-2/X-383-	L-2/X-384-
	L-2/X-385-	L-2/X-386-	L-2/X-387-	L-2/X-388-	L-2/X-389-	L-2/X-390-
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	L-2/X-415-	L-2/X-416-	L-2/X-417-	L-2/X-418-		
	L-3/X-1-	L-3/X-2-	L-3/X-3-	L-3/X-4-	L-3/X-5-	L-3/X-6-
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	L-3/X-13-	L-3/X-14-	L-3/X-15-	L-3/X-16-	L-3/X-17-	L-3/X-18-
	L-3/X-19-	L-3/X-20-	L-3/X-21-	L-3/X-22-	L-3/X-23-	L-3/X-24-
	L-3/X-25-	L-3/X-26-	L-3/X-27-	L-3/X-28-	L-3/X-29-	L-3/X-30-
	L-3/X-31-	L-3/X-32-	L-3/X-33-	L-3/X-34-	L-3/X-35-	L-3/X-36-
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	L-3/X-55-	L-3/X-56-	L-3/X-57-	L-3/X-58-	L-3/X-59-	L-3/X-60-
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25	L-3/X-67-	L-3/X-68-	L-3/X-69-	L-3/X-70-	L-3/X-71-	L-3/X-72-
	L-3/X-73-	L-3/X-74-	L-3/X-75-	L-3/X-76-	L-3/X-77-	L-3/X-78-
	L-3/X-79-	L-3/X-80-	L-3/X-81-	L-3/X-82-	L-3/X-83-	L-3/X-84-
	L-3/X-85-	L-3/X-86-	L-3/X-87-	L-3/X-88-	L-3/X-89-	L-3/X-90-

	L-3/X-91-	L-3/X-92-	L-3/X-93-	L-3/X-94-	L-3/X-95-	L-3/X-96-
	L-3/X-97-	L-3/X-98-	L-3/X-99-	L-3/X-100-	L-3/X-101-	L-3/X-102-
	L-3/X-103-	L-3/X-104-	L-3/X-105-	L-3/X-106-	L-3/X-107-	L-3/X-108-
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	L-3/X-133-	L-3/X-134-	L-3/X-135-	L-3/X-136-	L-3/X-137-	L-3/X-138-
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	L-3/X-181-	L-3/X-182-	L-3/X-183-	L-3/X-184-	L-3/X-185-	L-3/X-186-
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	L-3/X-265-	L-3/X-266-	L-3/X-267-	L-3/X-268-	L-3/X-269-	L-3/X-270-
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	L-3/X-331-	L-3/X-332-	L-3/X-333-	L-3/X-334-	L-3/X-335-	L-3/X-336-
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	L-3/X-355-	L-3/X-356-	L-3/X-357-	L-3/X-358-	L-3/X-359-	L-3/X-360-
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	L-4/X-151-	L-4/X-152-	L-4/X-153-	L-4/X-154-	L-4/X-155-	L-4/X-156-
	L-4/X-157-	L-4/X-158-	L-4/X-159-	L-4/X-160-	L-4/X-161-	L-4/X-162-



	L-4/X-163	L-4/X-164	L-4/X-165	L-4/X-166	L-4/X-167	L-4/X-168
	L-4/X-169	L-4/X-170	L-4/X-171	L-4/X-172	L-4/X-173-	L-4/X-174-
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	L-4/X-205-	L-4/X-206-	L-4/X-207-	L-4/X-208-	L-4/X-209-	L-4/X-210-
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10	L-4/X-217-	L-4/X-218-	L-4/X-219-	L-4/X-220-	L-4/X-221-	L-4/X-222-
	L-4/X-223-	L-4/X-224-	L-4/X-225-	L-4/X-226-	L-4/X-227-	L-4/X-228-
	L-4/X-229-	L-4/X-230-	L-4/X-231-	L-4/X-232-	L-4/X-233-	L-4/X-234-
	L-4/X-235-	L-4/X-236-	L-4/X-237-	L-4/X-238-	L-4/X-239-	L-4/X-240-
	L-4/X-241-	L-4/X-242-	L-4/X-243-	L-4/X-244-	L-4/X-245-	L-4/X-246-
15	L-4/X-247-	L-4/X-248-	L-4/X-249-	L-4/X-250-	L-4/X-251-	L-4/X-252-
	L-4/X-253-	L-4/X-254-	L-4/X-255-	L-4/X-256-	L-4/X-257-	L-4/X-258-
	L-4/X-259-	L-4/X-260-	L-4/X-261-	L-4/X-262-	L-4/X-263-	L-4/X-264-
	L-4/X-265-	L-4/X-266-	L-4/X-267-	L-4/X-268-	L-4/X-269-	L-4/X-270-
	L-4/X-271-	L-4/X-272-	L-4/X-273-	L-4/X-274-	L-4/X-275-	L-4/X-276-
20	L-4/X-277-	L-4/X-278-	L-4/X-279-	L-4/X-280-	L-4/X-281-	L-4/X-282-
	L-4/X-283-	L-4/X-284-	L-4/X-285-	L-4/X-286-	L-4/X-287-	L-4/X-288-
	L-4/X-289-	L-4/X-290-	L-4/X-291-	L-4/X-292-	L-4/X-293-	L-4/X-294-
	L-4/X-295-	L-4/X-296-	L-4/X-297-	L-4/X-298-	L-4/X-299-	L-4/X-300-
	L-4/X-301-	L-4/X-302-	L-4/X-303-	L-4/X-304-	L-4/X-305-	L-4/X-306-
25	L-4/X-307-	L-4/X-308-	L-4/X-309-	L-4/X-310-	L-4/X-311-	L-4/X-312-
	L-4/X-313-	L-4/X-314-	L-4/X-315-	L-4/X-316-	L-4/X-317-	L-4/X-318-
	L-4/X-319-	L-4/X-320-	L-4/X-321-	L-4/X-322-	L-4/X-323-	L-4/X-324-
	L-4/X-325-	L-4/X-326-	L-4/X-327-	L-4/X-328-	L-4/X-329-	L-4/X-330-
	L-4/X-331-	L-4/X-332-	L-4/X-333-	L-4/X-334-	L-4/X-335-	L-4/X-336-

L-4/X-337- L-4/X-338- L-4/X-339- L-4/X-340- L-4/X-341- L-4/X-342-  
 L-4/X-343- L-4/X-344- L-4/X-345- L-4/X-346- L-4/X-347- L-4/X-348-  
 L-4/X-349- L-4/X-350- L-4/X-351- L-4/X-352- L-4/X-353- L-4/X-354-  
 L-4/X-355- L-4/X-356- L-4/X-357- L-4/X-358- L-4/X-359- L-4/X-360-  
 5 L-4/X-361- L-4/X-362- L-4/X-363- L-4/X-364- L-4/X-365- L-4/X-366-  
 L-4/X-367- L-4/X-368- L-4/X-369- L-4/X-370- L-4/X-371- L-4/X-372-  
 L-4/X-373- L-4/X-374- L-4/X-375- L-4/X-376- L-4/X-377- L-4/X-378-  
 L-4/X-379- L-4/X-380- L-4/X-381- L-4/X-382- L-4/X-383- L-4/X-384-  
 L-4/X-385- L-4/X-386- L-4/X-387- L-4/X-388- L-4/X-389- L-4/X-390-  
 10 L-4/X-391- L-4/X-392- L-4/X-393- L-4/X-394- L-4/X-395- L-4/X-396-  
 L-4/X-397- L-4/X-398- L-4/X-399- L-4/X-400- L-4/X-401- L-4/X-402-  
 L-4/X-403- L-4/X-404- L-4/X-405- L-4/X-406- L-4/X-407- L-4/X-408-  
 L-4/X-409- L-4/X-410- L-4/X-411- L-4/X-412- L-4/X-413- L-4/X-414-  
 L-4/X-415- L-4/X-416- L-4/X-417- L-4/X-418-

### Pharmaceutical Formulations

When employed as pharmaceuticals, the compounds of Formula I are usually administered in the form of pharmaceutical compositions. This invention therefore provides pharmaceutical compositions which contain, as the active ingredient, one or more of the compounds of Formula I above or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable excipients, carriers, diluents, permeation enhancers, solubilizers and adjuvants. The compounds may be administered alone or in combination with other therapeutic agents (e.g., other antihypertensive drugs, diuretics and the like). Such compositions are prepared in a manner well known in the pharmaceutical art (*see, e.g., Remington's Pharm. Sci.*, Mack Publishing Co., Philadelphia, PA, 17<sup>th</sup> Ed. (1985) and "Modern Pharm.", Marcel Dekker, Inc., 3<sup>rd</sup> Ed. (G.S. Banker & C.T. Rhodes, Eds.)).

The compounds of Formula I may be administered by any of the accepted modes of administration of agents having similar utilities, for example, by oral, parenteral, rectal,

buccal, intranasal or transdermal routes. The most suitable route will depend on the nature and severity of the condition being treated. Oral administration is a preferred route for the compounds of this invention. In making the compositions of this invention, the active ingredient is usually diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders. Pharmaceutically acceptable salts of the active agents may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, e.g., by J. March, *Advanced Organic Chem. Reactions, Mechanisms and Structure*, 4<sup>th</sup> Ed. (N.Y.: Wiley-Interscience, 1992).

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents.

The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art. Controlled release drug delivery systems for oral administration include osmotic pump systems and dissolutional systems containing polymer-coated reservoirs or drug-polymer matrix formulations. Examples of controlled release systems are given in U.S. Patent Nos. 3,845,770; 4,326,525; 4,902,514; and 5,616,345. Another preferred

formulation for use in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. *See, e.g.*, U.S. Patent Nos. 5,023,252; 4,992,445 and 5,001,139. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

The compositions are preferably formulated in a unit dosage form. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient (e.g., a tablet, capsule, ampoule). The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. Preferably, for oral administration, each dosage unit contains from 1-250 mg of a compound of Formula I, and for parenteral administration, preferably from 0.1 to 60 mg of a compound of Formula I or a pharmaceutically acceptable salt thereof. It will be understood, however, that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules.

5 The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

10 The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

15 Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

5	<u>Ingredient</u>	Quantity
		(mg/capsule)
	Active Ingredient	30.0
	Starch	305.0
	Magnesium stearate	5.0

10 The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

15	<u>Ingredient</u>	Quantity
		(mg/tablet)
	Active Ingredient	25.0
	Cellulose, microcrystalline	200.0
20	Colloidal silicon dioxide	10.0
	Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

25 Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

<u>Ingredient</u>	<u>Weight %</u>
Active Ingredient	5
Lactose	95

5           The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

10

<u>Ingredient</u>	<u>Quantity (mg/tablet)</u>
Active Ingredient	30.0
Starch	45.0
15   Microcrystalline cellulose	35.0
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0
Sodium carboxymethyl starch	4.5
Magnesium stearate	0.5
Talc	1.0
20   Total	120.0

25

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50°C to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

5	<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
	Active Ingredient	40.0
	Starch	109.0
	Magnesium stearate	1.0
	Total	150.0

10

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

15      Suppositories, each containing 25 mg of active ingredient are made as follows:

<u>Ingredient</u>	<u>Amount</u>
Active Ingredient	25.0 mg
Saturated fatty acid glycerides to	2,000.0 mg

20

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

25

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:



<u>Ingredient</u>	<u>Amount</u>
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
5 Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 ml

10

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then

15 added to produce the required volume.

#### Formulation Example 8

<u>Ingredient</u>	<u>Quantity</u> <u>(mg/capsule)</u>
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

25

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

### Formulation Example 9

A subcutaneous formulation may be prepared as follows:

	<u>Ingredient</u>	<u>Quantity</u>
5	Active Ingredient	5.0 mg
	Corn Oil	1.0 mL

10 Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472 which is herein incorporated by reference.

15 Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

### Synthesis Examples

25

#### Example 1. (Figure 7A)

Preparation of N,N'-dimethyl-N,N'-di-[2-[4-[2-butyl-3-benzofuranylcarbonyl]-2,6-diiodophenoxy]ethyl]hexane, (7A-2), in which n=1, and Link is (CH<sub>2</sub>)<sub>6</sub>.

A. A solution of 3-[(2-bromoethoxy)-3,5-diodobenzoyl]-2-butylbenzofuran (7A-1), prepared as described in *Eur. J. Med. Chem.*, 1974, 19-25, and in Belgian Patent 900138, (2 mmol), diisopropylethylamine (5 mmol) and 1,6-di-(methylamino)hexane (1 mmol) in acetonitrile (25mL) is maintained at room temperature, and the reaction is monitored by thin layer chromatography (tlc). When it is complete, the solution is added to water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 7A-2.

B. In a similar manner, by employing different diamines in place of 1,6-di-(methylamino)hexane, as described herein, in A above, different compounds of Formula 7A-2 are obtained.

C. In similar manner, by employing different bromo compounds of Formula 7A-1, as described herein, in A above, different compounds of Formula 7A-2 are obtained.

#### Example 2. (Figure 7B)

Preparation of 1,8-di-[2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethyl]methylamino]-3,5-dioxaoctane, 7B-2, in which n is 1, and Link is  $(\text{CH}_2)_2(\text{O}(\text{CH}_2)_2)_2$ .

A. A solution of N-methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]-ethylamine (7B-1), prepared according to procedures described in *Eur. J. Med. Chem.*, 1974, 19-25, (5 mmol), 1,8-dibromo-3,5-dioxaoctane (2.5 mmol) and diisopropylethylamine (2mL) in EtOH (25mL) is maintained at room temperature. The progress of the reaction is followed by TLC. When it is complete, the mixture is poured into water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 7B-2, in which n is 1 and Link is  $(\text{CH}_2)_2(\text{O}(\text{CH}_2)_2)_2$ .

B. In a similar manner, by employing different compounds 7B-1, as described herein, different linked compounds of Formula 7B-2 are obtained.

C. In a similar manner, by employing different linking compounds, as described herein, in place of 1,8-dibromo-3,5-dioxaoctane, different linked compounds of Formula 7B-2 are obtained.

### Example 3. (Figure 7C)

Preparation of 1,10-di-[2-butyl-[3-[4-(3-dibutylaminopropoxy)benzoyl]benzofuran-5-yl]aminosulfonyl]decane, 7C-2, in which Link is  $((CH_2)_2)_{10}$ .

A. 5-Amino-2-butyl-3-[4-(3-dibutylaminopropoxy)benzoyl]benzofuran (7C-1), prepared as described in EP 0471609, (1 mmol) and 1,10-di(chlorosulfonyl)decane (0.5 mmol) are heated at reflux in  $CH_2Cl_2$  (20mL). The progress of the reaction is followed by tlc. When it is complete, the solution is added to dilute  $Na_2CO_3$ . The organic phase is separated, dried and evaporated, and the residue is chromatographed to afford the title compound 7C-2.

B. In a similar manner, by employing different di-(chlorosulfonyl) linking compounds, as described herein, different linked compounds of Formula 7C-2 are obtained

### Example 4. (Figure 8A)

Preparation of 1,6-di[4-[2-[2-[4-(methylsulfonylamino)phenoxy]-ethylmethylamino]ethyl]phenylaminosulfonyl]hexane 8A-2, in which Link is  $(CH_2)_4$ .

A. 2-[4-(Methylsulfonylamino)phenoxy]ethyl bromide, 21-1, (10 mmol) and N-methyl 2-(4-nitrophenyl)ethylamine, 21-2, (10 mmol) both prepared as described in *J. Med. Chem.*, 1990, 1151, are heated at reflux in MeCN (100mL) containing  $K_2CO_3$  (3g) and KI (0.2g). The reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is

chromatographed to afford N-methyl N-(4-nitrophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]ethylamine (21-3).

5 B. The above compound (3mmol) is dissolved in EtOH (50mL) and Raney nickel (1g) is added. The mixture is stirred in a hydrogen atmosphere. The progress of the reaction is monitored by tlc. When it is complete, the solution is filtered and then evaporated. The residue is chromatographed to afford N-methyl N-(4-aminophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]ethylamine, 8A-1.

10 C. A solution of hexane-1,6-disulfonyl chloride (1 mmol), diisopropylethylamine (1mL) and 8A-1 (0.5 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (25mL) is maintained at room temperature. The progress of the reaction is monitored by tlc. When it is complete, the solvent is removed under reduced pressure and the residue is chromatographed to afford the title compound 8A-2, in which Link is  $(\text{CH}_2)_4$ .

15 D. In a similar manner, by employing different di(chlorosulfonyl) linking compounds, as described herein, in C above, different linked compounds 8A-2 are prepared.

#### Example 5. (Figure 8B)

20 Preparation of 1,4-di[-4-[2-methyl-2-[4-(methylsulfonylamino)phenyl]ethylamino]-ethoxy]phenyl]aminosulfonylmethyl]benzene, 8B-2, where Link is  $p\text{-CH}_2\text{C}_6\text{H}_4\text{CH}_2$ .

A. 2-(4-Nitrophenoxy)ethyl bromide, 21-4, (10 mmol) and 2-(4-methylsulfonylamino)phenyl N-methylethylamine 21-5 (10 mmol) are heated at reflux in MeCN (50mL) containing  $\text{K}_2\text{CO}_3$  (2g). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford N-methyl N-(4-(methylsulfonyl-amino)phenylethyl) 2-(4-aminophenoxy)ethylamine 8B-1.

B. The above compound **8B-1**, (1 mmol) and 1,4-di-(chlorosulfonylmethyl)benzene (0.5 mmol) are dissolved in  $\text{CH}_2\text{Cl}_2$  (50 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is washed with dilute  $\text{Na}_2\text{CO}_3$ , then dried and evaporated. The residue is chromatographed to afford **8B-2**, in which Link is  $p\text{-CH}_2\text{C}_6\text{H}_4\text{CH}_2$ .

C. In a similar manner, by employing different di(sulfonyl chloride) linking compounds, as described herein, in B above, different linked compounds **8B-2** are prepared

Example 6. (Figure 8C)

Preparation of 1,10-di-[2-[4-(methylsulfonylaminophenoxy)ethyl] 2-[4-[methylsulfonylaminophenyl]ethyl]amino]decane, **8C-2**, in which Link is  $(\text{CH}_2)_{10}$ .

A. N-Benzyl 2-[4-(methylsulfonylaminophenyl)]ethylamine, (**21-7** prepared as described in EP 338793), (10 mmol), 2-[4-(methylsulfonylaminophenoxy)ethyl bromide **21-8** (10 mmol) and  $\text{K}_2\text{CO}_3$  (1 g) are heated at reflux in MeCN (50 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford N-benzyl N-2-(4-methylsulfonylaminophenoxy)ethyl 2-(4-methylsulfonylaminophenyl)ethylamine, **21-9**.

B. The compound **21-9** (1 mmol) is dissolved in EtOH (20 mL) and ammonium formate (100 mg) and 10% Pd/C (50 mg) are added. The progress of the reaction is monitored by tlc. When it is complete, the solution is filtered then added to water and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford N-[2-(4-methylsulfonylaminophenoxy)ethyl] 2-(4-methylsulfonylaminophenyl)-ethylamine, **8C-1**.

C. The above compound (1mmol), 1,10-dibromodecane (0.5 mmol),  $K_2CO_3$  (1g) and KI (0.05g) are heated at reflux in MeCN. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated. The residue is chromatographed to afford the title compound 8C-2, in which Link is  $(CH_2)_{10}$ .

D. In a similar manner, by employing different dialkylating agents, as described herein, in place of 1,10-dibromodecane, in C above, different compounds of Formula 8C-2 are obtained.

#### Example 7. (Figure 9A)

Preparation of 1,8-di-[4-[4-(ethylheptylamino)-1-hydroxybutyl]phenylamino-sulfonyl]octane, 9A-2, in which Link is  $(CH_2)_6$ .

A. 4-Nitrophenyl-4-oxobutanoic acid, 21-10, prepared as described in *Gazz. Chim. Ital.*, 1967, 97, 654, and U.S. Patent 5,405,851 (10 mmol), 1-hydroxybenztriazole (10 mmol) and dicyclohexylcarbodiimide (10 mmol) are dissolved in DMF (50mL). To the solution is added ethylheptylamine (10 mmol). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford N-ethyl N-heptyl 4-(4-nitrophenyl)-4-oxobutanamide, 21-11.

B. The above prepared compound (2 mmol) is dissolved in dry diglyme (20mL) and MeOH (1mL). Lithium borohydride (25 mmol) is added and the solution is heated to reflux. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford 4-[1-hydroxy-4(ethylheptylamino)butyl]aniline 9A-1.

C. The compound 9A-1 (1 mmol) and octane-1,8-disulfonyl chloride (0.5 mmol) are dissolved in  $\text{CH}_2\text{Cl}_2$  (25mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute  $\text{Na}_2\text{CO}_3$ , and then extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 9A-2., in which Link is  $(\text{CH}_2)_6$ .

D. In a similar manner, by employing different disulfonyl chlorides, as described herein, in C above, different compounds of Formula 9A-2 are obtained.

Example 8. (Figure 9B)

Preparation of 1,6-di-[4-[4-hydroxy-4-(methylsulfonylaminophenyl)butyl]-ethylamino]hexane 9B-2, in which Link is  $(\text{CH}_2)_6$ .

A. 4-(4-Aminophenyl)-4-oxobutanoic acid 21-12, (5 mmol) is added to a solution of dicyclohexylcarbodiimide (5 mmol) and ethylamine (5 mmol) in THF (50mL). After 12 hours, the mixture is added to water and extracted with EtOAc. The extract is washed with dilute NaOH, then dried and evaporated. The residue is chromatographed to afford N-ethyl 4-(4-aminophenyl)-4-oxobutanamide, 21-13.

B. The product from A above (3 mmol) is dissolved in THF (25mL) and to the solution is added diisopropylethylamine (5 mmol) and methanesulfonyl chloride (3 mmol). The reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford N-ethyl 4-(4-methylsulfonylaminophenyl)-4-oxobutanamide, 21-14.

C. The above-described compound (1 mmol) is dissolved in ether (50mL); the solution is cooled to  $0^\circ\text{C}$ , and to it is added lithium aluminum hydride (5 mmol). The reaction is monitored by tlc. When it is complete, excess hydride is decomposed by addition



When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound, 20-9, in which X is O.

5           B.     In a similar manner, by employing N-methyl 2-[(4-methylsulfonylamino)phenyl]-ethylamine, (20-6, in which X is a direct bond) there is obtained the corresponding product 1,3,5-tri-[2-[4-(methylsulfonylamino)phenyl]ethylmethylamino-methyl]benzene, 20-9, in which X is a direct bond.

10           While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular  
15           situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

20           All of the publications, patent applications and patents cited in this application are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

Table 1: Multiple Activities of Antiarrhythmic Agents

Drug	Channels					Receptors		
	Na			Ca	K	$\alpha$	$\beta$	M <sub>2</sub>
	Fast	Med.	Slow					
<b>Class I</b>								
Lidocaine	•							
Mexiletine	•							
Tocainide	•							
Moricizine	♦							
Procainamide		♦			■			
Disopyramide		♦			■			•
Quinidine		♦			■	•		•
Propafenone		♦					■	
Flecainide			♦		•			
Encainide			♦					
<b>Class IV</b>								
Bepridil	•			♦	■			
Verapamil	•			♦		■		
Diltiazem				■				
<b>Class III</b>								
Bretylium					♦	★	★	
Sotalol					♦		♦	
Amiodarone	•			•	♦	■	■	
Afinidine					■			

Potency of block: • Low    ■ Moderate    ♦ High    ★ Biphasic Action

Table 2: Activity of Potassium Channel Ligands

Drug	Development Stage	I <sub>kr</sub>	I <sub>Ks</sub>	I <sub>to</sub>	Na <sub>slow</sub>	Use Dependence
Amiodarone	Approved	•	•			None
Dofetilide	Phase 3	•				Reverse
Sematilide	Phase 3	•				Reverse
E-4031	Phase 2	•				Reverse
Azimilide	Phase 3	•	•			Reverse
Ambasilide	Terminated	•	•			None
Ibutilide	Approved	•			1	None
Tedisamil	Phase 3	•		•		Reverse

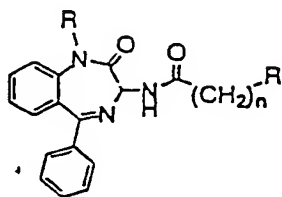
Table 3: Properties of Potassium-Channel Blockers

Drug	Trade Name	Developer	Status	IC50 ( $\mu$ M)			Bioavail.	Elim $t_{1/2}$
				IK <sub>v</sub>	IK <sub>r</sub>	I <sub>o</sub>		
Dofetilide	Tikosyn	Pfizer	Phase 3	0.01			100%	7-13 hrs.
E-4031		Eisai	Phase 2	0.4			90%	
Sematilide		Schering AG	Phase 3	25			50%	
Ambasilide		BASF	Term.					
Azimilide	Stedcor	P&G	Phase 3	0.2	2			
Tedisamil		Solvay	Phase 3	<10		5		
Dronedaron		Sanofi	Phase 2					
Ibutilide	Corvert	Pharmacia	Apprvd	0.016			<5%	4-8 hrs.
d-Sotalol		Bristol-Myers	Pre-reg	100			100%	
Amiodarone	Cordarone	Wyeth-Ayerst	Apprvd				22-86%	3-21 hrs.

Table 4: Potassium Channel Ligands

UK66,914 (I <sub>K</sub> )			
RP52866 (I <sub>K1</sub> )		Amiodarone	
Tacrine (I <sub>K</sub> and I <sub>K1</sub> )			
4-Aminopyridine (I <sub>to</sub> )			
Glibenclamide (I <sub>K(ATP)</sub> )			
RP49356 (I <sub>K(ATP)</sub> opener)			
	<u>N-Acetyl Procainamide (NAPA)</u>		
	<u>Clofilium</u>		

Table 4: Potassium Channel Ligands (continued)

Effect of Substitution on  $I_K$  PotencyL - 768,673  
and analogs

compd <sup>a</sup>	abs conf	R	n	R'	$I_K$ IC <sub>50</sub> (nM) <sup>b</sup>	$I_K$ IC <sub>50</sub> (nM) <sup>c</sup> or % inh at concn (nM)	CCK-B IC <sub>50</sub> (nM) <sup>d</sup>	mp °C
2 <sup>20</sup>	R	Me	0	<i>m</i> -NHPhCH <sub>3</sub>	214	5000	2 ± 0.3	
2b <sup>20</sup>	S	Me	0	<i>m</i> -NHPhCH <sub>3</sub>	10000	10000	151 ± 12	
3	R	Me	0	phenyl	600 <sup>d</sup>	23% at 1000	>1000	224-225
4	R	Me	0	3,5-dichlorophenyl	45 <sup>d</sup>	17% at 1000	>1000	179-180
5	R	Me	1	phenyl	300 <sup>d</sup>	31% at 1000	>1000	241-242
6	R	Me	1	2,4-dichlorophenyl	35			209-210
7	R	Me	1	2,4-bis(trifluoromethyl)phenyl	140			100-103
8	R	Me	2	phenyl	200 <sup>d</sup>	32% at 1000	>1000	179
9	R	Me	2	2,4-dichlorophenyl	14 <sup>d</sup>	31% at 100	>1000	92-95
10	R	Me	0	CH=CH-2,4-dichlorophenyl	6 <sup>d</sup>	1500	>1000	137-139
11	R	Me	0	CH(NH <sub>2</sub> )CH <sub>3</sub> Ph	2800 <sup>d</sup>	8800	>1000	84-86
12	R	Me	2	4-aminophenyl	4400 <sup>d</sup>		>1000	175-178
13	R	Me	2	4-acetamidophenyl	>10000		>1000	138-142
14	R	Me	2	cyclohexyl	10 <sup>d</sup>	1000	>1000	144-145
15	RS	H	2	cyclohexyl	1000			192-193
16	RS	Me <sub>2</sub> NCH <sub>2</sub> CH <sub>3</sub>	2	2,4-dichlorophenyl	520 <sup>d</sup>	100	>1000	199-201
17	R	<i>i</i> -Pr	2	cyclohexyl	20 <sup>d</sup>		>1000	154-155
18	R	<i>i</i> -Pr	0	3,5-dichlorophenyl	6			140-141
19	S	<i>i</i> -Pr	0	3,5-dichlorophenyl	110	6% at 1000	>1000	140-141
20	R	<i>i</i> -Pr	1	3,5-dichlorophenyl	10 <sup>d</sup>			90-96
21	R	F <sub>2</sub> CCH <sub>3</sub>	0	3,5-dichlorophenyl	11 <sup>d</sup>	25% at 100		140-143
22	R	F <sub>2</sub> CCH <sub>3</sub>	1	3,5-dichlorophenyl	30	4000		93-100
23	R	F <sub>2</sub> CCH <sub>3</sub>	1	2,4-dichlorophenyl	9	2400		143-145
24	S	F <sub>2</sub> CCH <sub>3</sub>	1	2,4-bis(trifluoromethyl)phenyl	60 <sup>d</sup>	35% at 1000	>1000	
1	R	F <sub>2</sub> CCH <sub>3</sub>	1	2,4-bis(trifluoromethyl)phenyl	6 <sup>d</sup>	6000	>1000	132-134

Table 5: Principal Cardiac K<sup>+</sup> Currents and Some Drugs that Block Them

Current	Drugs that Block Current	Reference
I <sub>K</sub>	UK66,914, dofetilide, sematilide, <i>d</i> -solatol	Argentieri, 1992; Carmeliet, 1985; Gwilt, et al., 1990, 1991
I <sub>K1</sub>	RP58866, RP62719	Escande, et al., 1992; Imoto, et al., 1987
I <sub>TO1</sub>	Tedisamil	Dukes, et al., 1990
I <sub>K(ATP)</sub>	Glibenclamide, 5-hydroxydecanoate	Kantor, et al., 1990; Notsu, et al., 1989; 1992

## WHAT IS CLAIMED IS:

1. A multibinding compound comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel.

2. The multibinding compound of Claim 1 wherein said ligand is selected from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide, ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

3. The multibinding compound of Claim 1 which has 2 ligands.

4. A multibinding compound represented by Formula I:



where each L is a ligand that may be the same or different at each occurrence;

X is a linker that may be the same or different at each occurrence;

p is an integer of from 2 to 10; and

q is an integer of from 1 to 20;

wherein each of said ligands comprises a ligand domain capable of binding to a K<sup>+</sup> channel.

5. The multibinding compound of Claim 4, wherein q is less than p.

6. The multibinding compound of Claim 4 wherein said ligand is selected from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium,



melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide, ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

7. The multibinding compound of Claim 4 wherein  $p$  is 2 and  $q$  is 1.

8. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of one or more multibinding compounds, or pharmaceutically acceptable salts thereof, comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a  $K^+$  channel of a cell mediating mammalian diseases or conditions, thereby modulating the diseases or conditions.

9. The pharmaceutical composition of Claim 8 wherein said ligand is selected from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide, ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

10. The pharmaceutical composition of Claim 8 which has 2 ligands.

11. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of one or more multibinding compounds represented by Formula I:



5 and pharmaceutically acceptable salts thereof,

where each L is a ligand that may be the same or different at each occurrence;

X is a linker that may be the same or different at each occurrence;

p is an integer of from 2 to 10; and

q is an integer of from 1 to 20;

10 wherein each of said ligands comprises a ligand domain capable of binding to a K<sup>+</sup> channel of a cell mediating mammalian diseases or conditions, thereby modulating the diseases or conditions.

12. The pharmaceutical composition of Claim 11 wherein said ligand is selected  
15 from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide,  
20 ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

13. The pharmaceutical composition of Claim 11 which has 2 ligands.

25 14. A method for modulating the activity of a K<sup>+</sup> channel in a biologic tissue, which method comprises contacting a tissue having a K<sup>+</sup> channel with a multibinding compound, or a pharmaceutically acceptable salt thereof, under conditions sufficient to produce a change in the activity of the channel in said tissue, wherein the multibinding compound comprises 2 to 10 ligands which may be the same or different and which are

covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel.

15        15.     The method of Claim 14 wherein said ligand is selected from the group  
consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium,  
melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide,  
almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-  
aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine,  
disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide,  
10    ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and  
amiodarone.

16.     The method of Claim 14 wherein the multibinding compound has 2 ligands.

15        17.     A method for treating a disease or condition in a mammal resulting from an  
activity of a K<sup>+</sup> channel, which method comprises administering to said mammal a  
therapeutically effective amount of a pharmaceutical composition comprising a  
pharmaceutically acceptable excipient and one or more multibinding compounds, or  
pharmaceutically acceptable salts thereof, comprising 2 to 10 ligands which may be the same  
20    or different and which are covalently attached to a linker or linkers, which may be the same  
or different, each of said ligands comprising a ligand domain capable of binding to a K<sup>+</sup>  
channel of a cell mediating mammalian diseases or conditions.

25        18.     The method of Claim 17 wherein said ligand is selected from the group  
consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium,  
melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide,  
almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-  
aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine,  
disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide,

ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

19. The method of Claim 17 wherein the multibinding compound has 2 ligands.

20. A method for treating a disease or condition in a mammal resulting from an activity of a  $K^+$  channel, which method comprises administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds represented by Formula I:



and pharmaceutically acceptable salts thereof,

where each L is a ligand that may be the same or different at each occurrence;

X is a linker that may be the same or different at each occurrence;

p is an integer of from 2 to 10; and

q is an integer of from 1 to 20;

wherein each of said ligands comprises a ligand domain capable of binding to a  $K^+$  channel of a cell mediating mammalian diseases or conditions.

21. The method of Claim 20 wherein said ligand is selected from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide, ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

22. The method of Claim 20 wherein the multibinding compound has 2 ligands.

23. A method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

5 (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

10 (d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

15 24. A method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

20 (b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

25 (d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

25. The method according to Claim 23 or 24 wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

5

26. The method according to Claim 25 wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

27. The method according to Claim 26 wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

10

28. The method according to Claim 27 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

15

29. The method according to Claim 23 or 24 wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

30. The method according to Claim 29 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

20

31. The method according to Claim 23 or 24 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and amphiphilic linkers.

25

32. The method according to Claim 31 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

33. The method according to Claim 32 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

34. The method according to Claim 23 or 24 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

35. The method according to Claim 34 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

36. The method according to Claim 23 or Claim 24 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

37. The method according to Claim 23 or Claim 24 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

38. A library of multimeric ligand compounds which may possess multivalent properties for potassium channels, which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the

library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

39. A library of multimeric ligand compounds which may possess multivalent properties for potassium channels, which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

40. The library according to Claim 38 or Claim 39 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and amphiphilic linkers.

41. The library according to Claim 40 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

42. The library according to Claim 41 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

43. The library according to Claim 38 or 39 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.



44. The library according to Claim 43 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive  
5 functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

45. The library according to Claim 38 or Claim 39 wherein the multimeric  
10 ligand compound library comprises homomeric ligand compounds.

46. The library according to Claim 38 or Claim 39 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

15 47. An iterative method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or  
20 mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

25 (b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

5 (e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted or are consistent with imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

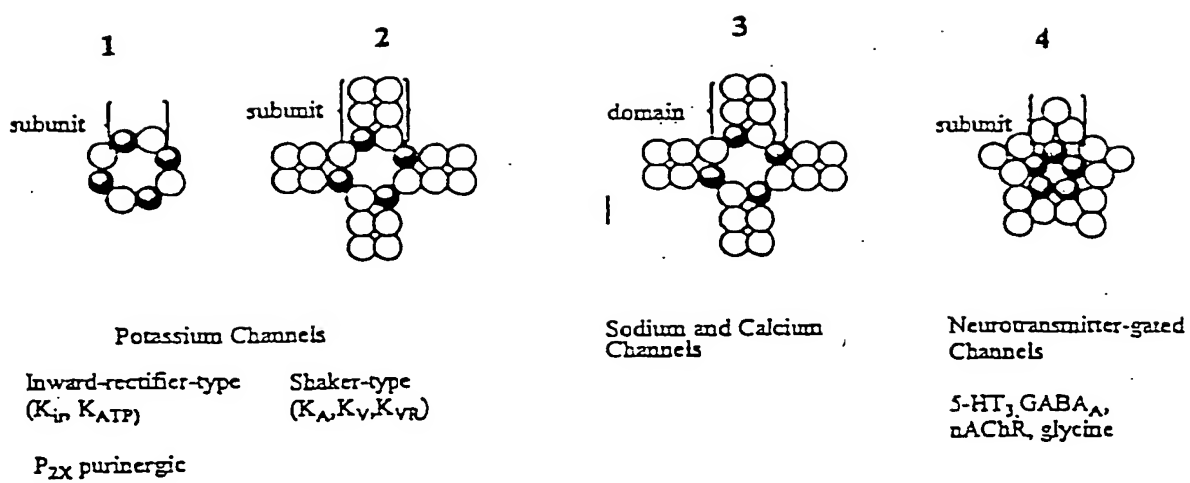
10 (g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

48. The method according to Claim 47 wherein steps (e) and (f) are repeated from 2-50 times.

15 49. The method according to Claim 47 wherein steps (e) and (f) are repeated from 5-50 times.

1/24

Figure 1



2/24

Figure 2A

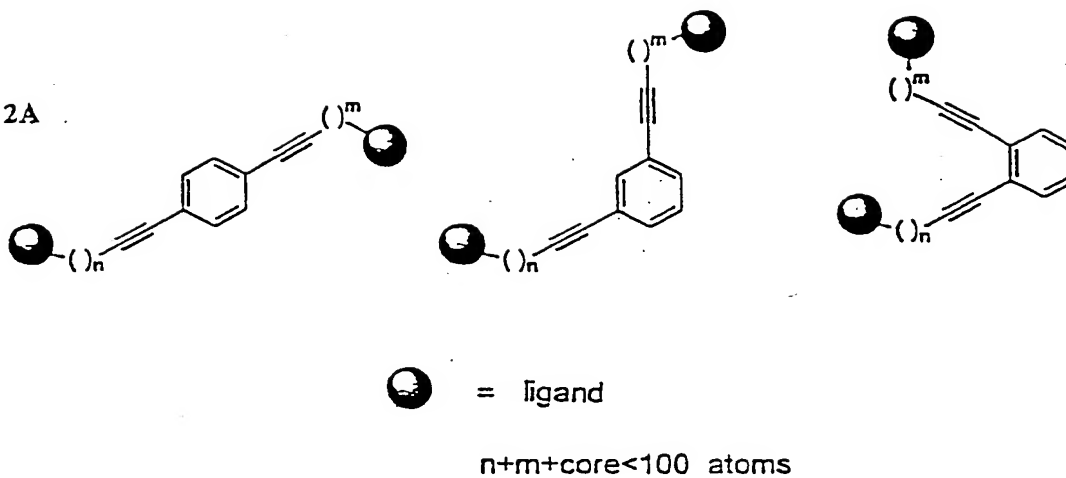


Figure 2B

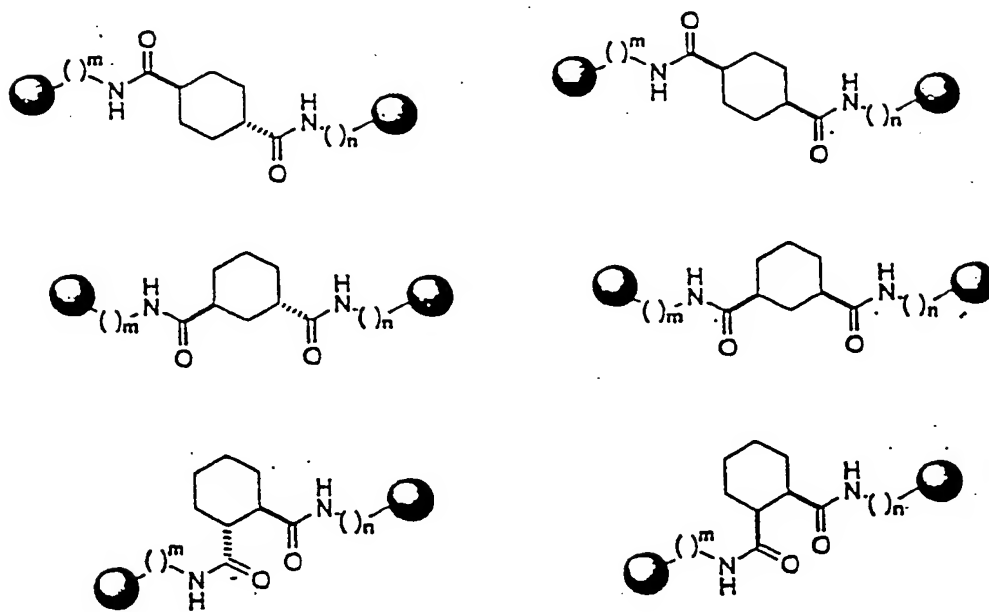
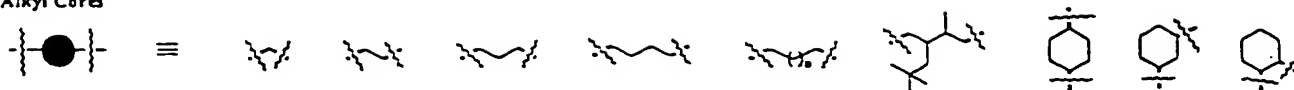
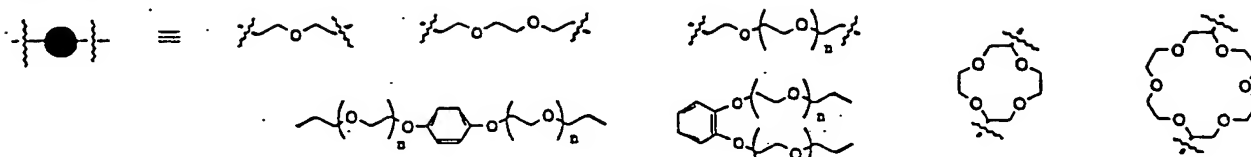


Figure 3

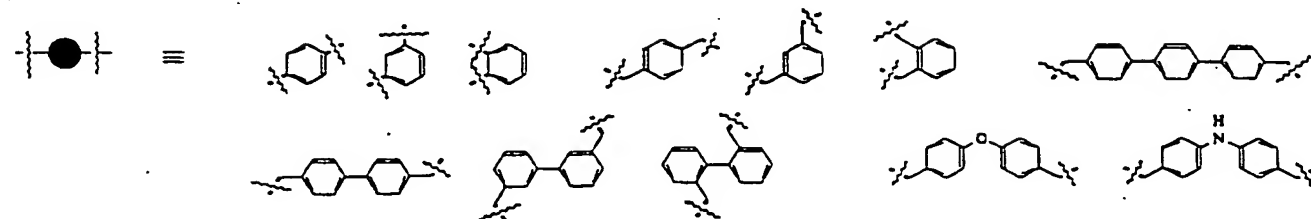
## Alkyl Cores



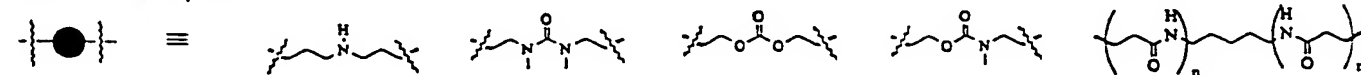
## Ether Cores



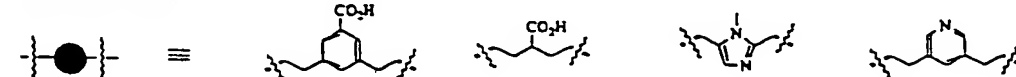
## Aromatic Cores



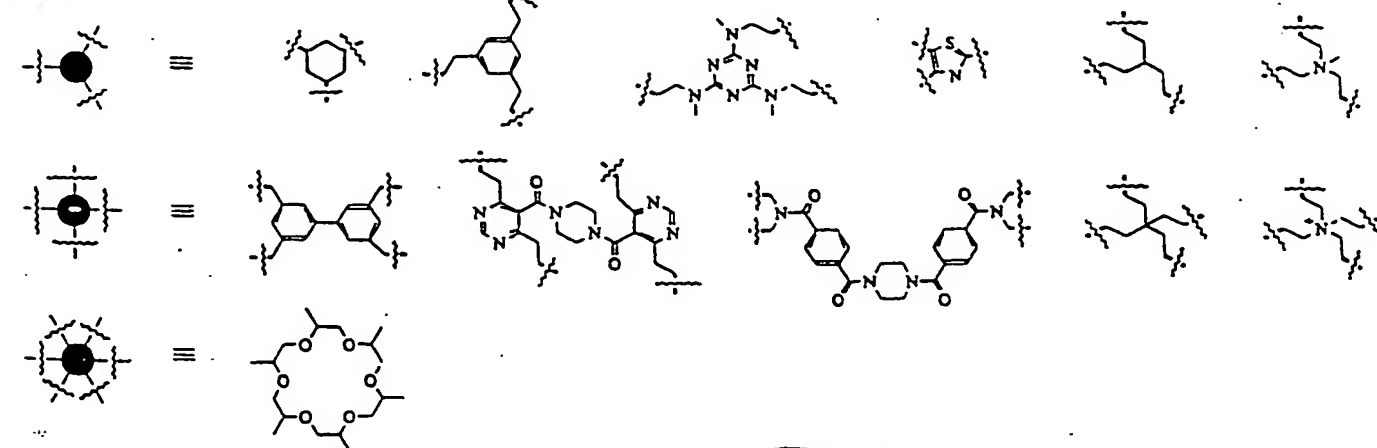
## H-bond Donor/Acceptor Cores



## Acidic/Basic Cores



## Higher Order Cores



## Peptidic Cores

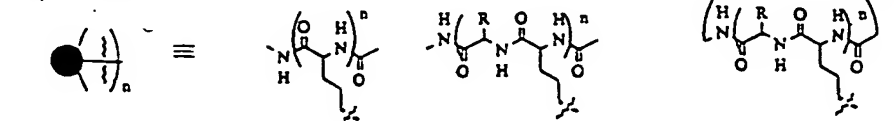


Figure 4A

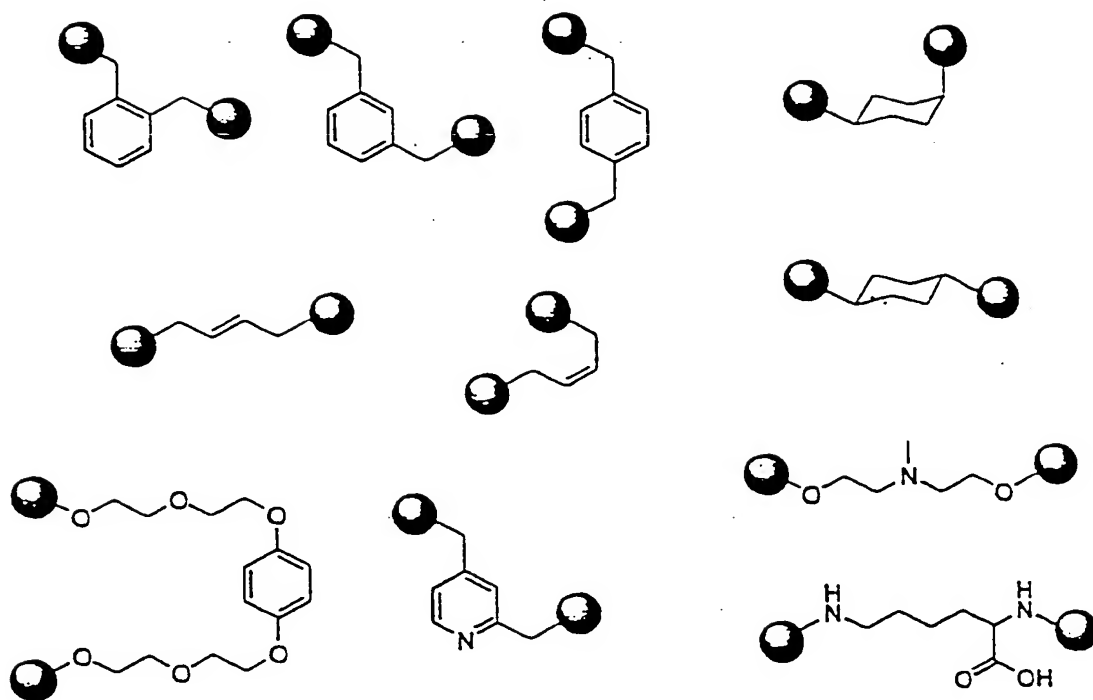
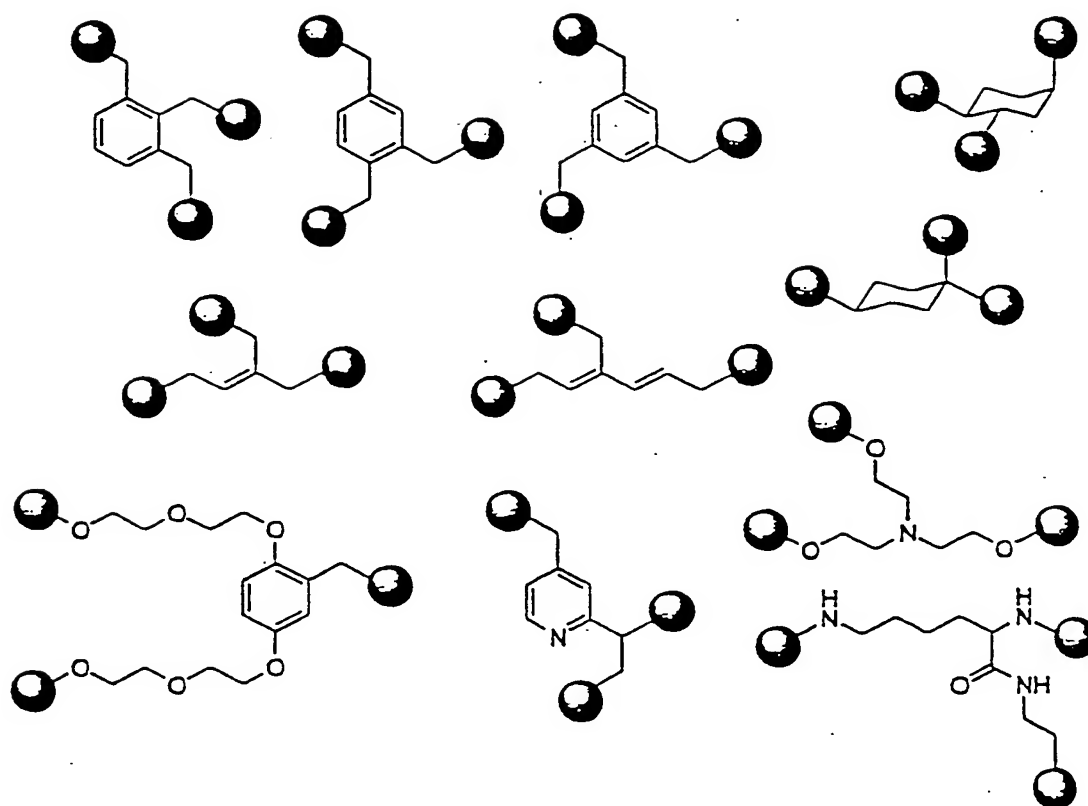
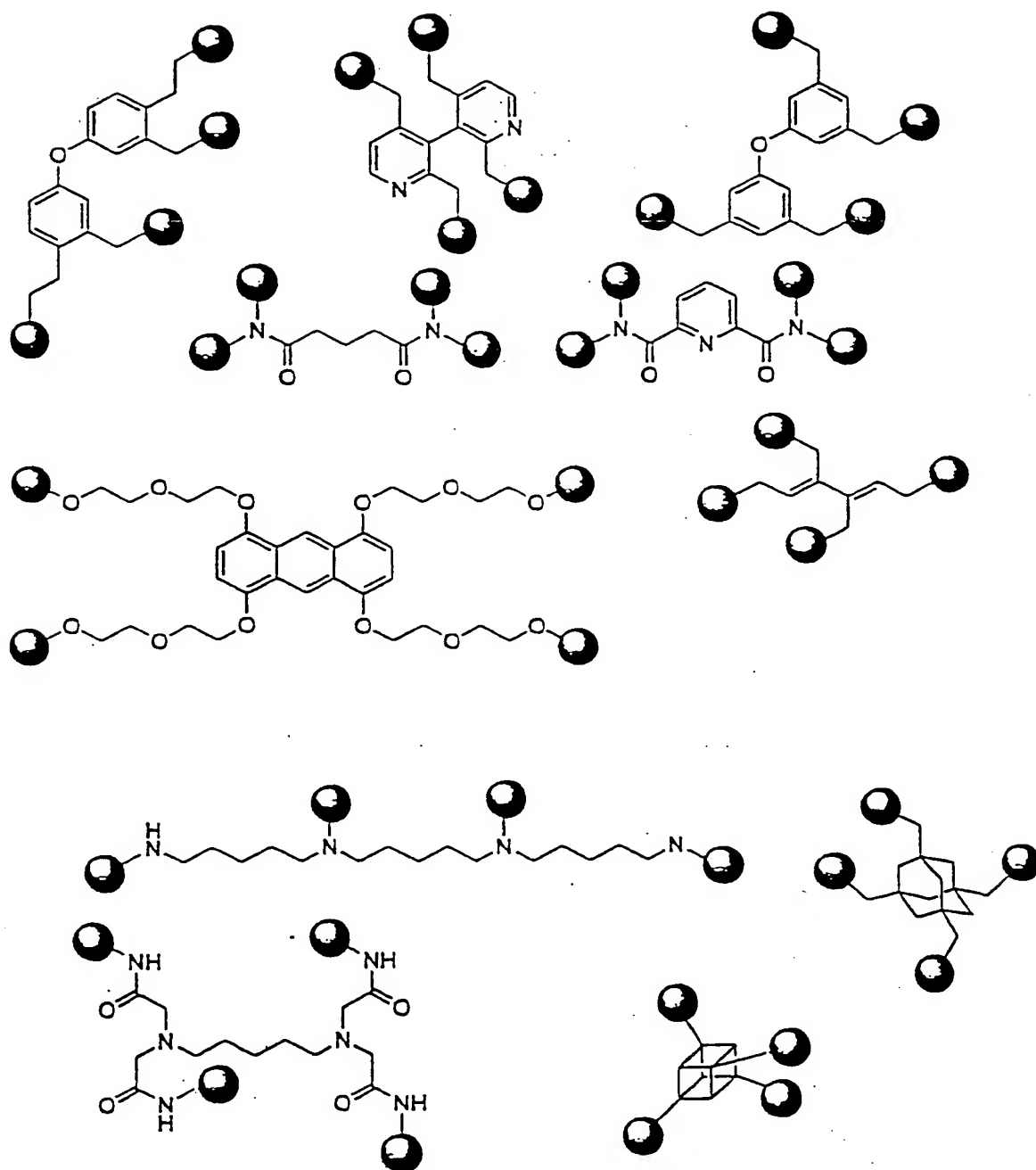


Figure 4B



6/24

Figure 4C





7/24

Figure 4D

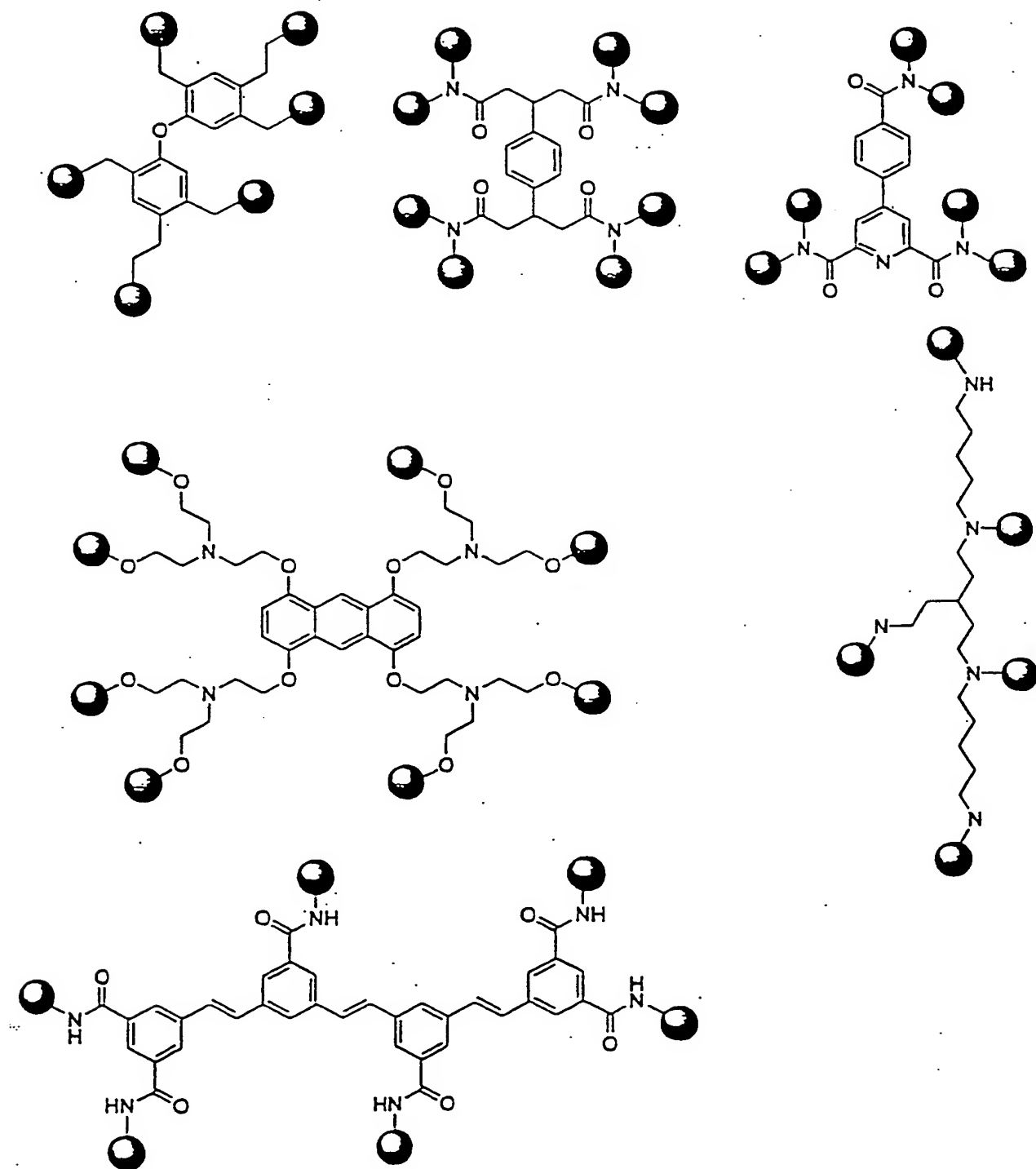
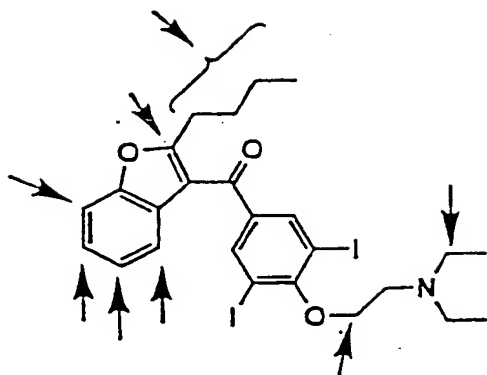
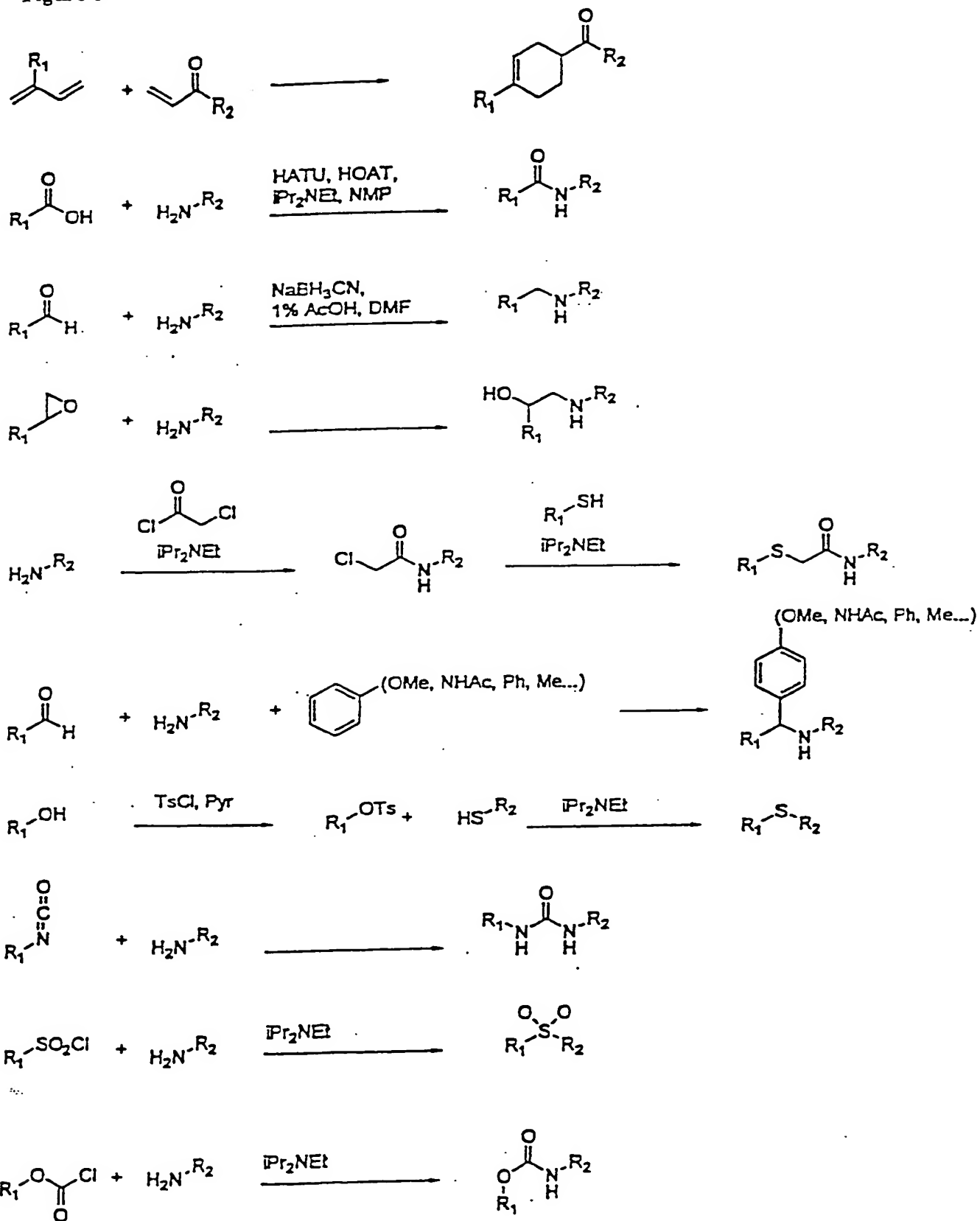


Figure 5



9/24

Figure 6



10/24

Figure 7A

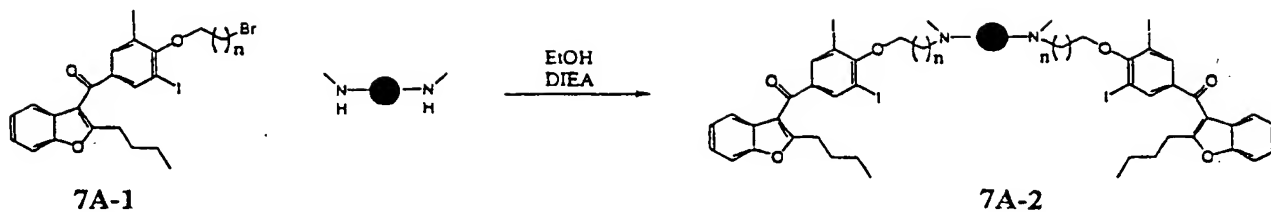


Figure 7B

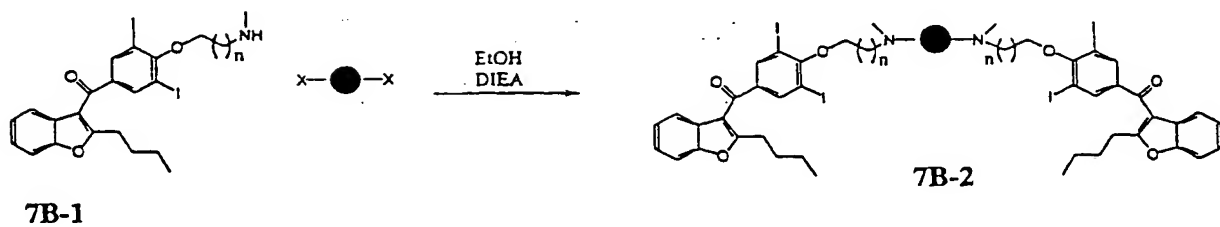


Figure 7C

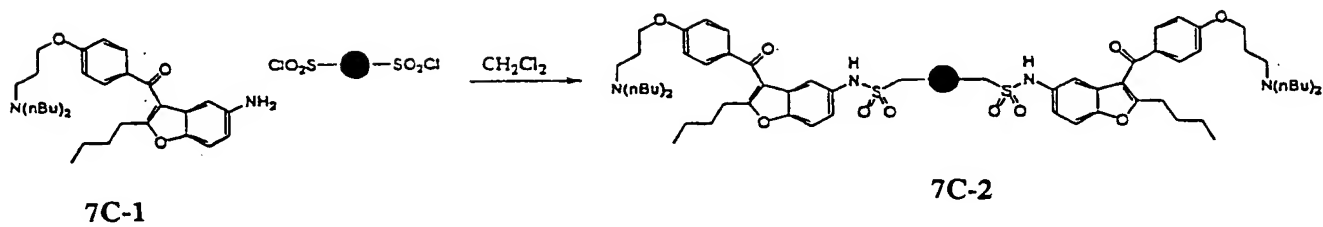


Figure 7. Reaction Schemes for Amiodarone and Dronedarone

11/24

Figure 8A

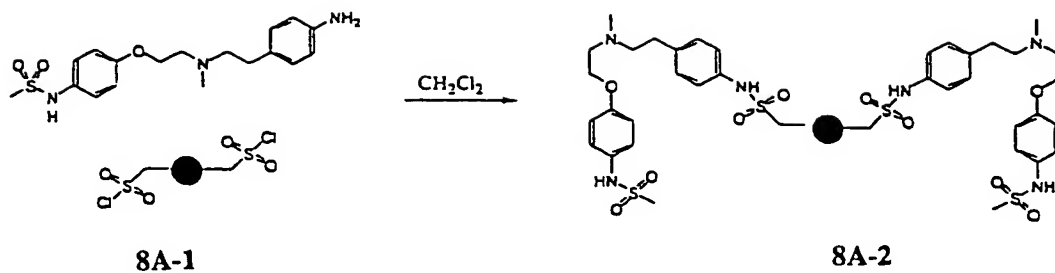


Figure 8B

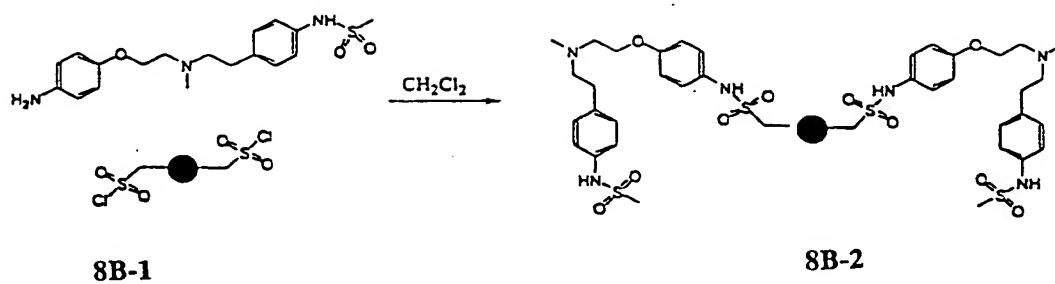


Figure 8C

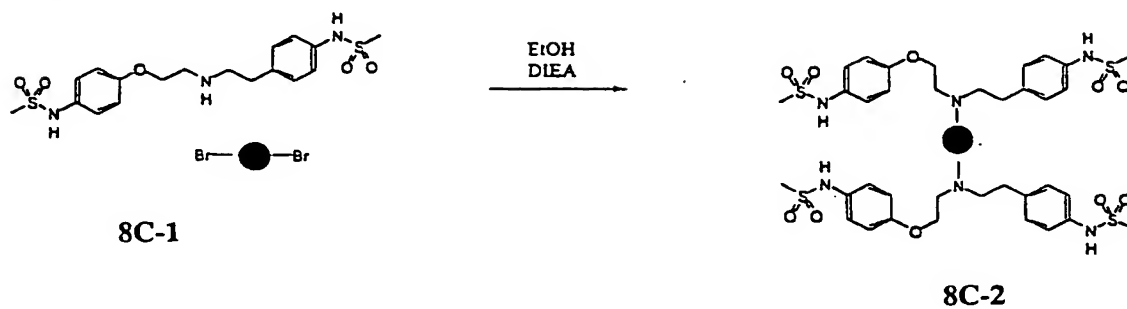


Figure 8. Reaction Schemes for Bivalent Dofetilide Compounds

12/24

Figure 9A

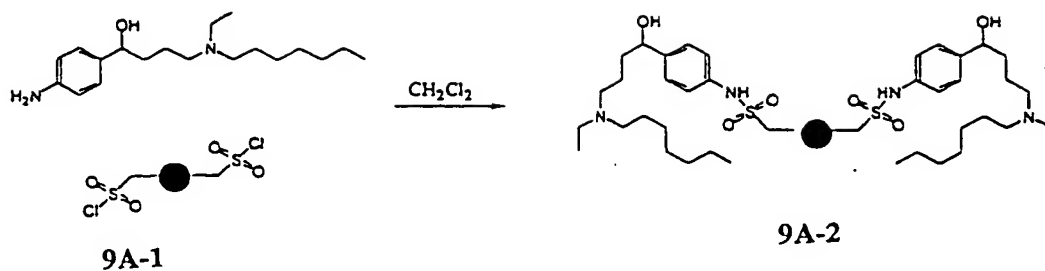


Figure 9B

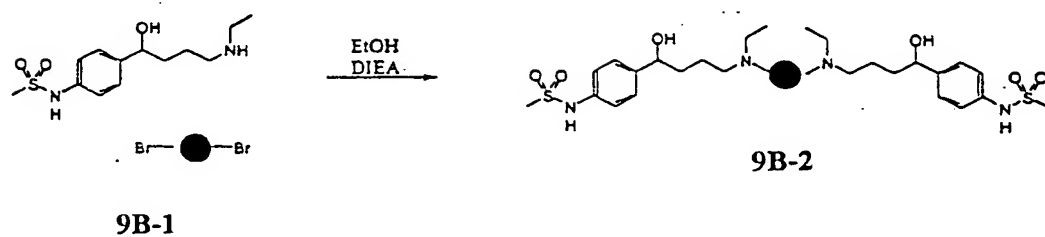


Figure 9. Reaction Schemes for Bivalent Ibutilide Compounds

13/24

Figure 10A

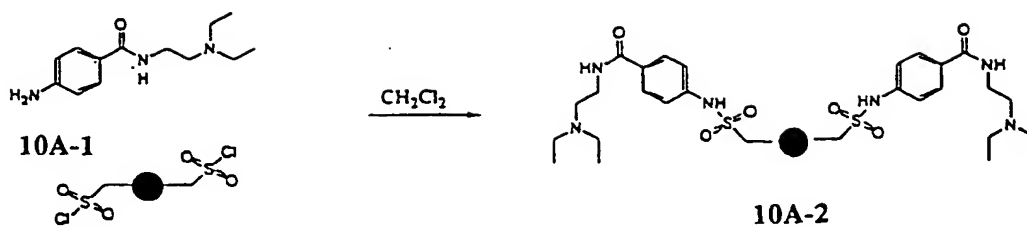


Figure 10B

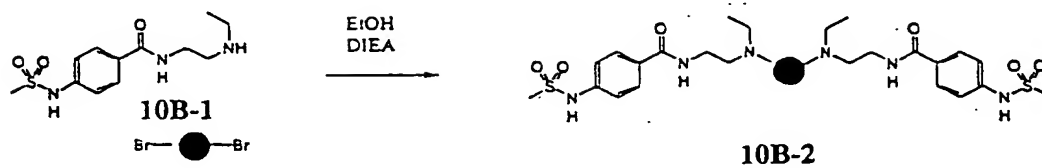


Figure 10. Reaction Schemes for Bivalent Sematilide Compounds

14/24

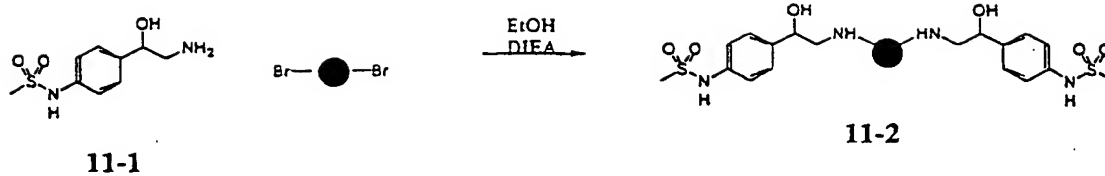


Figure 11. Reaction Schemes for Bivalent Sotalol Compounds



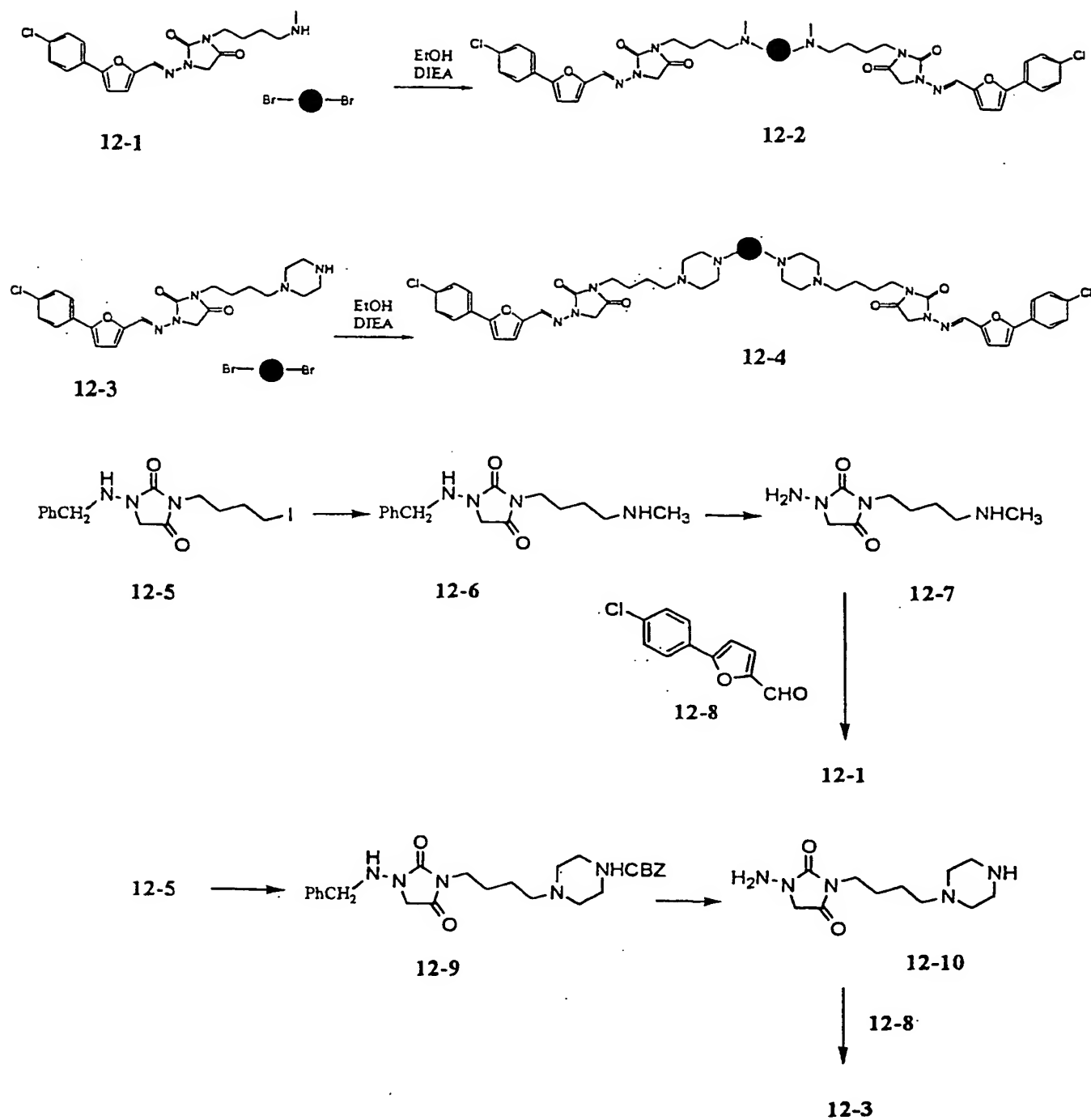


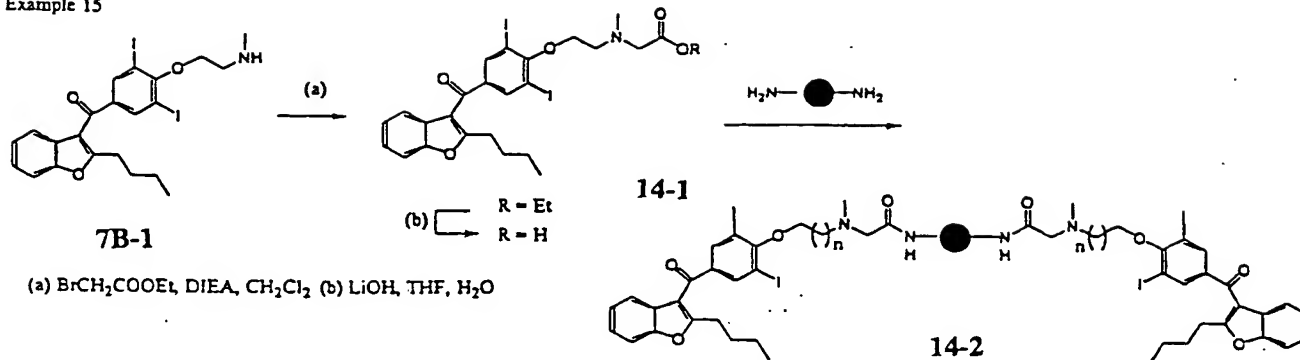
Figure 12. Reaction Schemes for Bivalent Azimilide Compounds

16/24

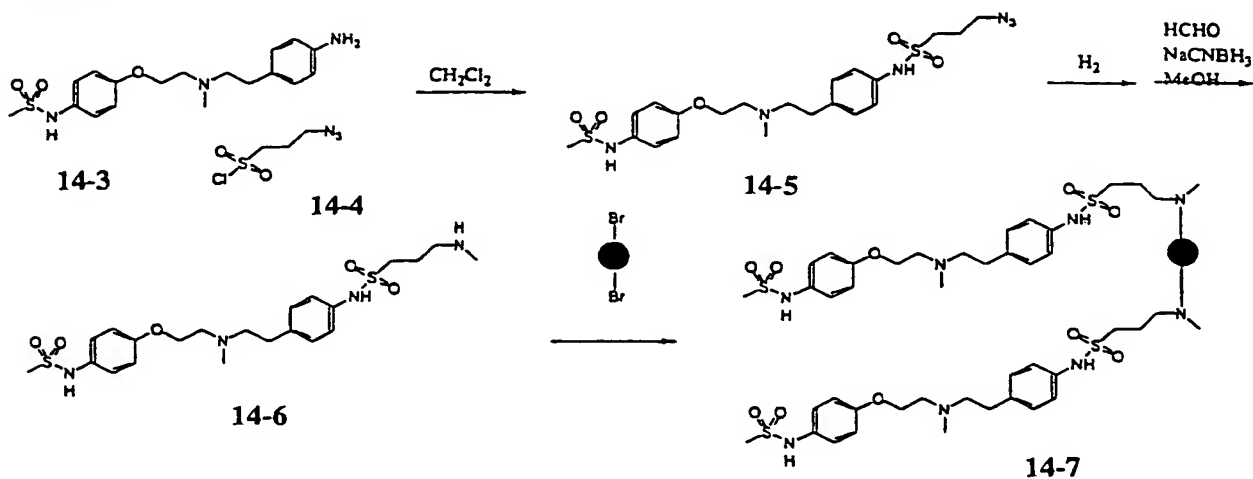


Figure 13. Reaction Schemes for Bivalent Tedasimil Compounds

## Example 15



## Example 16



## Example 17

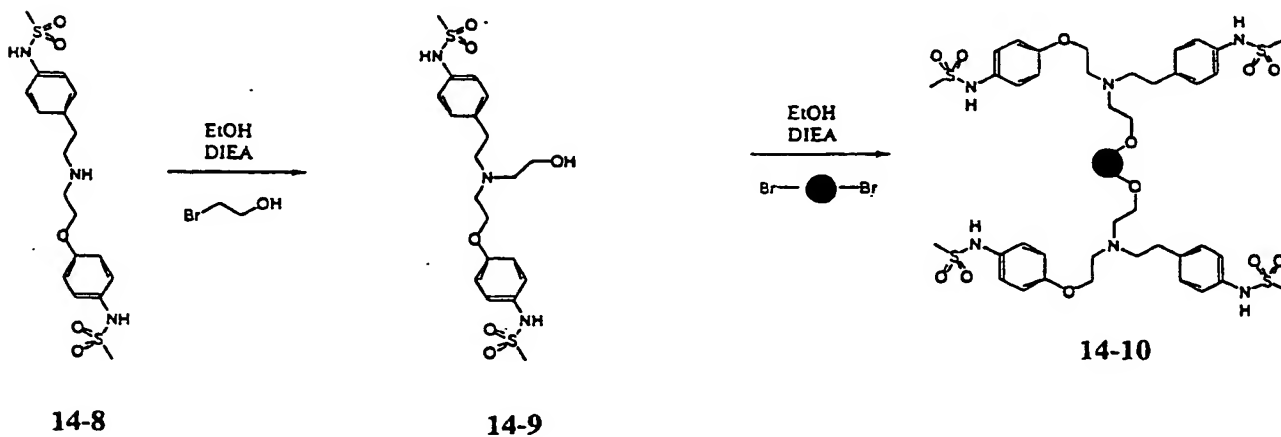
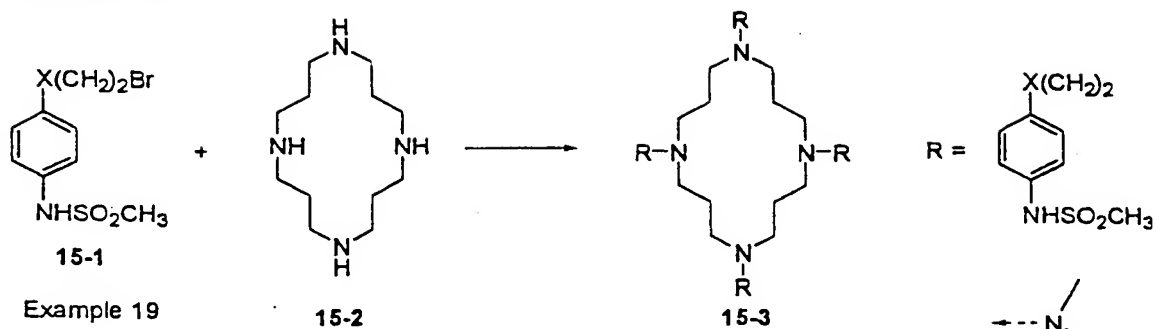


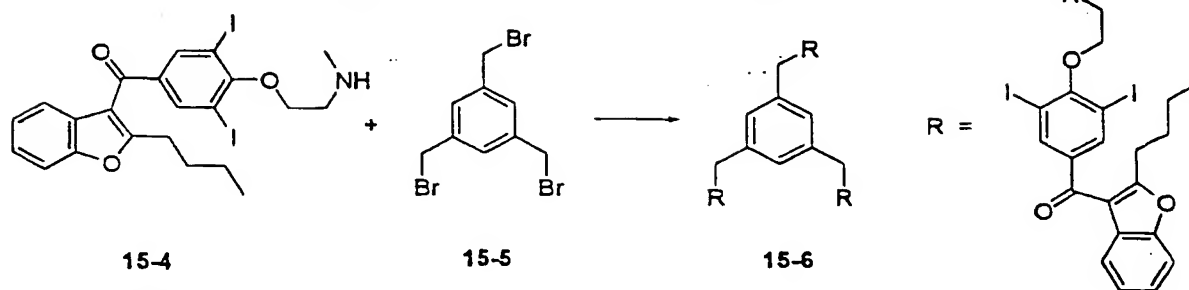
Figure 14. Introduction of Spacer to Facilitate Multivalomer Formation

18/24

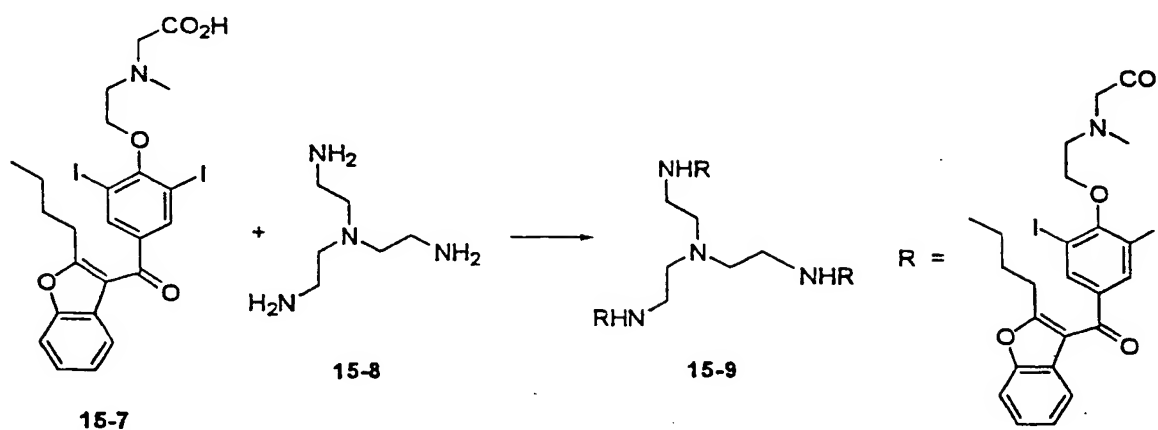
## Example 18



## Example 19



## Example 20



## Example 21

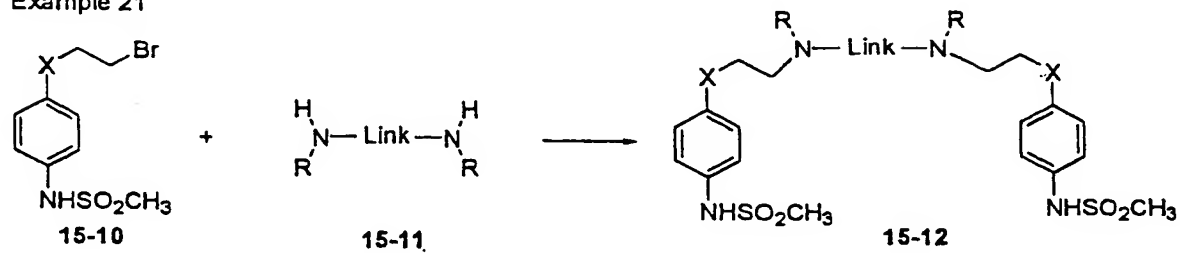


FIGURE 15

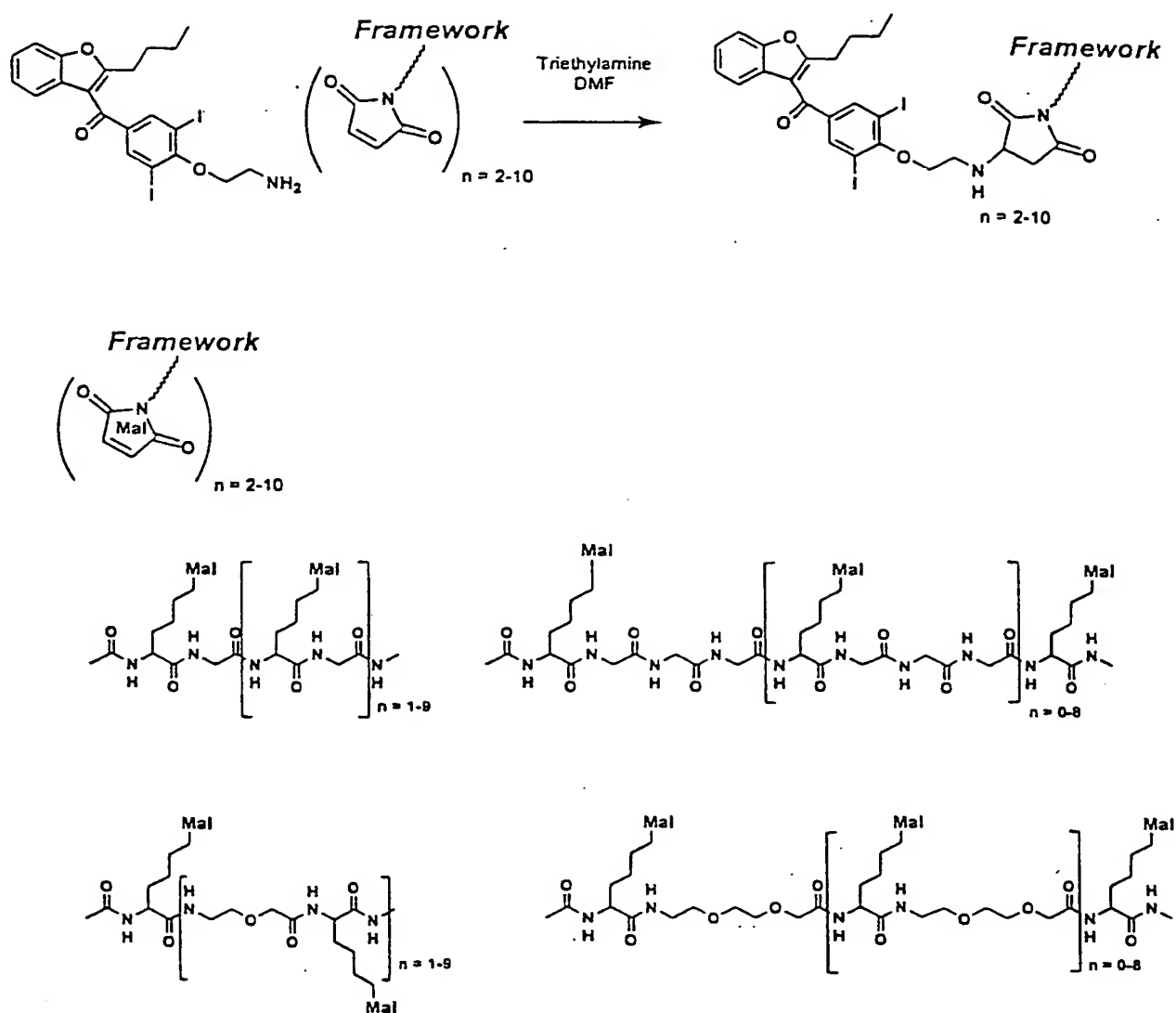


Figure 16. Multimers with Peptide Core and Maleimide Coupling

20/24

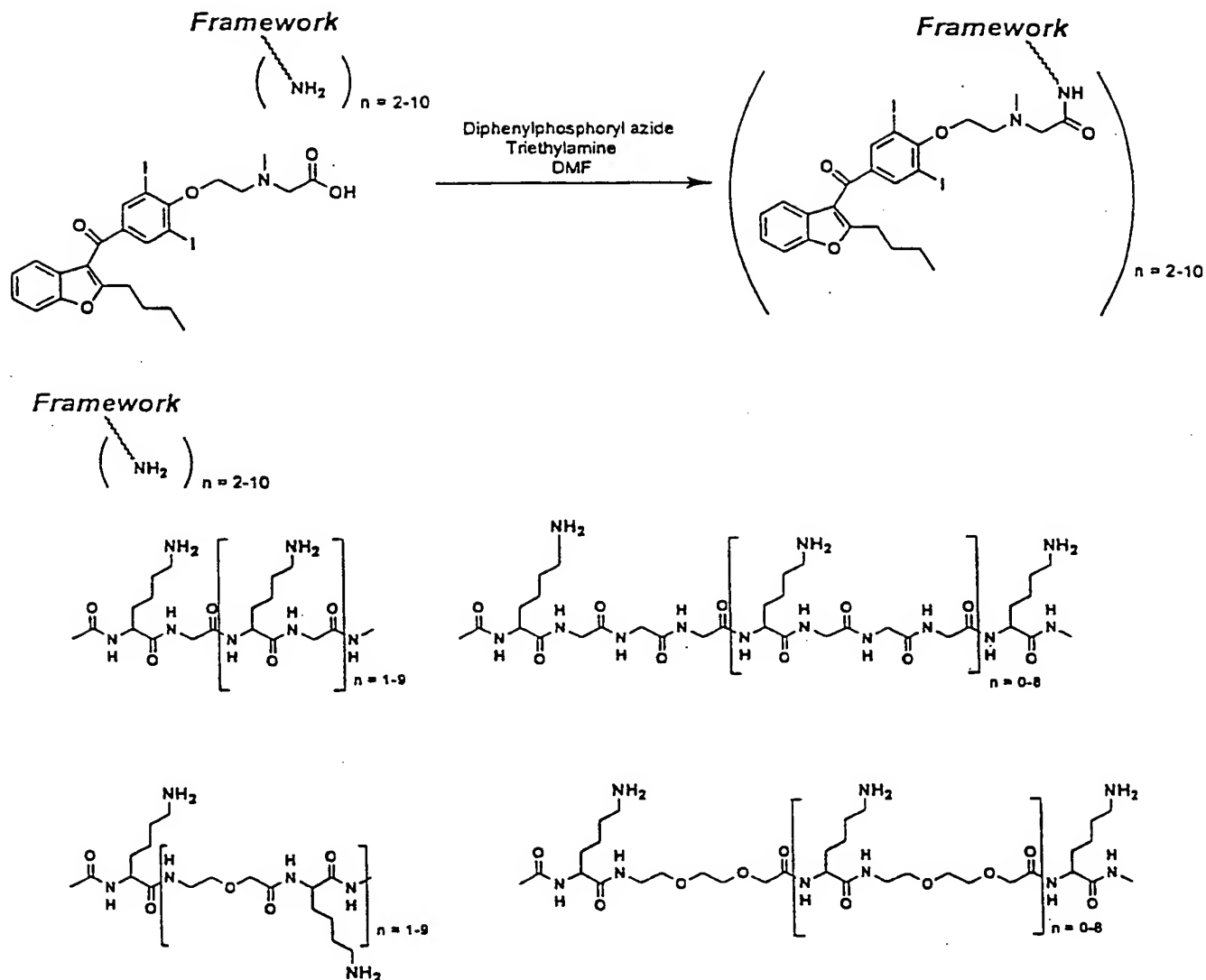


Figure 17. Polypeptide Cores

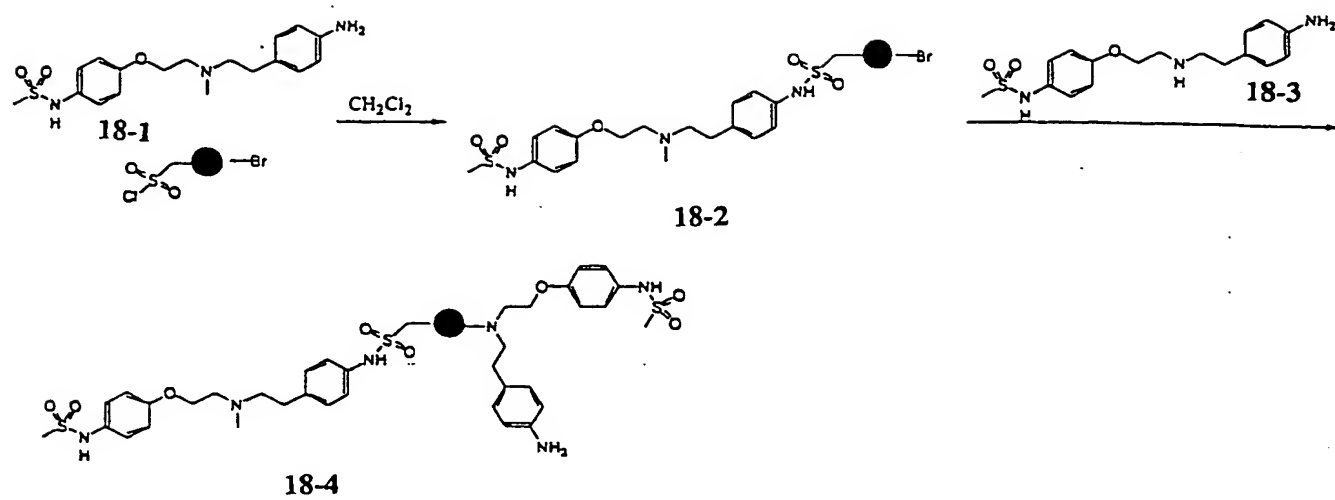
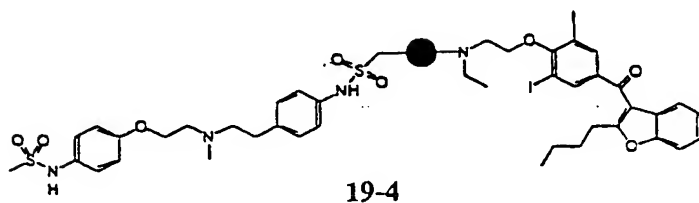
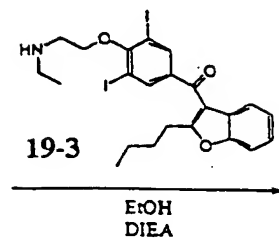
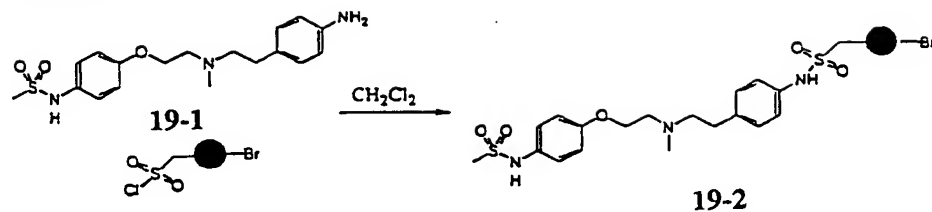
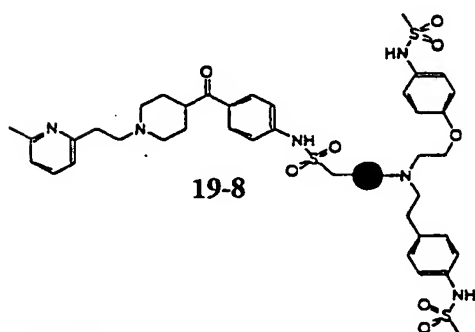
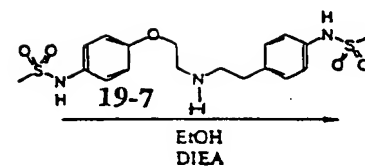
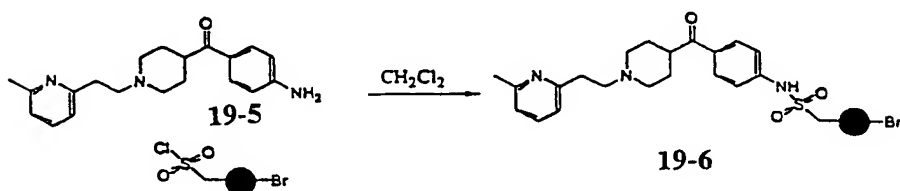


Figure 18. Assymetric Linear Bivalent Compound

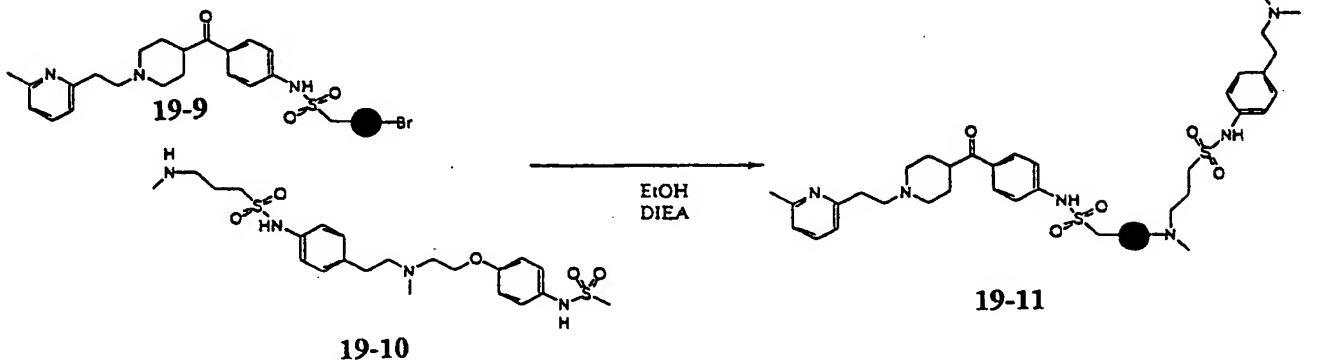
### Example 23



### Example 24



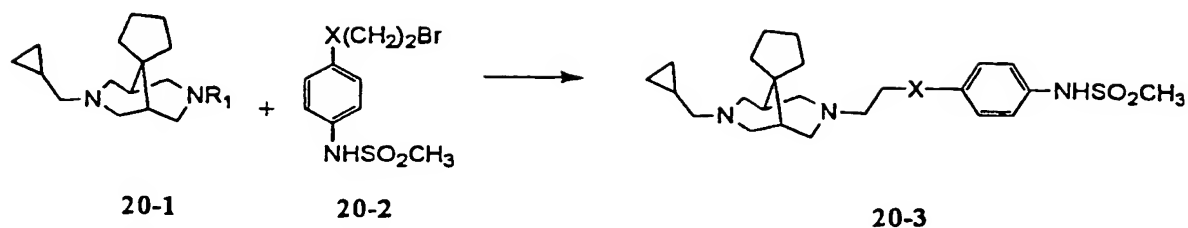
### Example 25



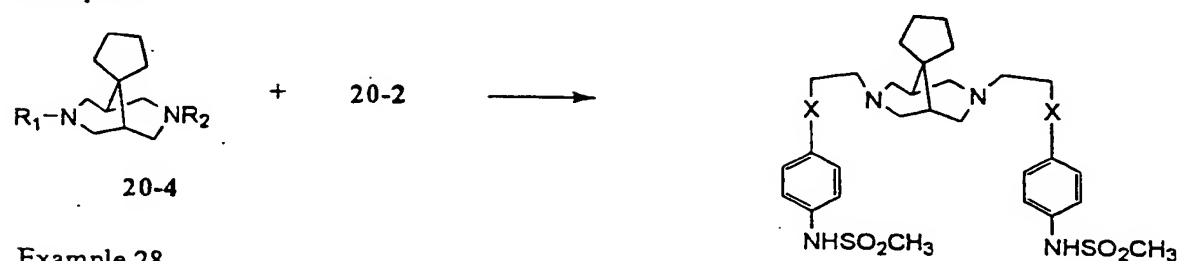
**Figure 19. Heterovalomers**



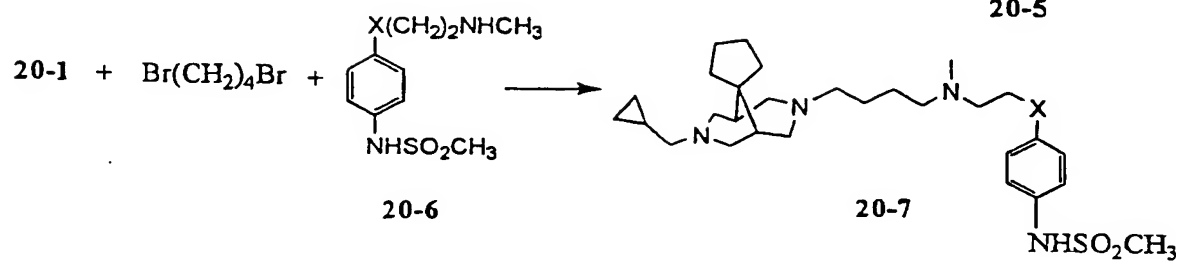
## Example 26



## Example 27



## Example 28



## Example 29

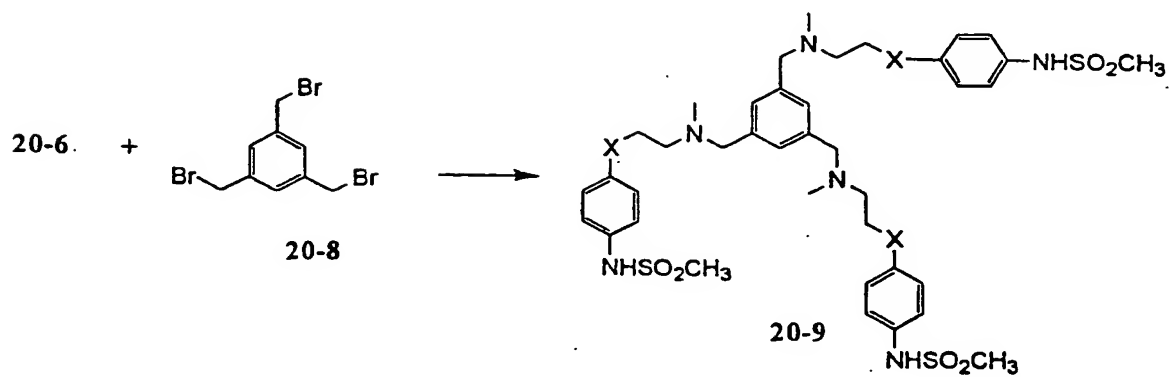


FIGURE 20

24/24

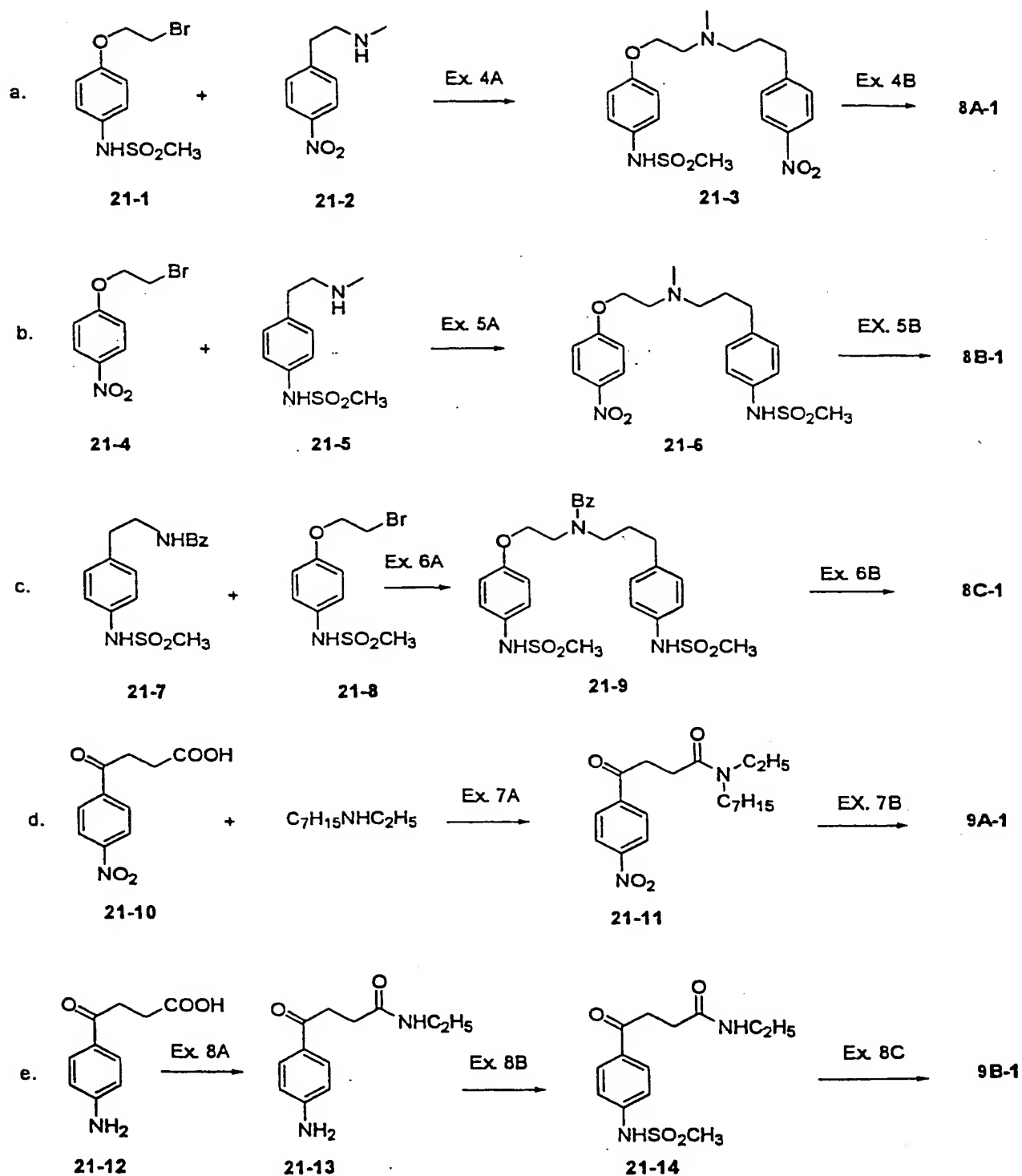


Figure 21. Preparation of Intermediates

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/12777

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN (CAPLUS, BIOSIS, EMBASE, MEDLINE, SCISEARCH)

Search Terms: potassium channel, multivalent, combinatorial, benzodia?

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/05802 A1 (NEORX CORPORATION) 16 April 1992 (16.04.92), see Abstract, page 3 lines 1-25, page 4 lines 20-27, page 5 lines 6-18, page 21 lines 4-33, page 22 lines 1-8 and claim 1.	1-49
Y	US 5,545,568 A (ELLMAN) 13 August 1996, see column 1 lines 21-53, column 6 lines 55-67 and column 7, lines 1-30.	1-49
Y	BUNIN et al. 'Synthesis and Evaluation of Three 1,4-Benzodiazepine Libraries.' In: Combinatorial Peptide and Nonpeptide Libraries, Edited by Gunther Jung, New York: VCH 1996, pages 405-424. See entire article, especially Introduction, page 405.	1-49

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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CHEMICAL MATRIX

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/12777

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	SALATA et al. A Novel Benzodiazepine that Activates Cardiac Slow Delayed Rectifier K <sup>+</sup> Currents. Molecular Pharmacology. July 1998, Vol. 53, pages 220-230. See entire article.	1-49
Y	SHUKER et al. Discovering High-Affinity Ligands for Proteins: SAR by NMR. Science. 29 November 1996, Vol. 274, pages 1531-1534. See entire article, especially Figure 1.	23-49

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/12777

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 38/00, 39/00, 39/44, 39/395, 51/00; C07K 2/00, 4/00; G01N 33/53, 33/543, 33/566

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807





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<b>(51) International Patent Classification 6 :</b> <b>A61K 38/00, 39/00, 39/44, 39/395, 51/00,</b> <b>C07K 2/00, 4/00, G01N 33/53, 33/543,</b> <b>33/566</b>	<b>A1</b>	<b>(11) International Publication Number: WO 99/64050</b> <b>(43) International Publication Date: 16 December 1999 (16.12.99)</b>
<b>(21) International Application Number:</b> PCT/US99/12777 <b>(22) International Filing Date:</b> 7 June 1999 (07.06.99)  <b>(30) Priority Data:</b> 60/088,465               8 June 1998 (08.06.98)       US 60/093,068            16 July 1998 (16.07.98)       US 60/113,864            24 December 1998 (24.12.98)   US  <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications</b> US                               60/088,465 (CON) Filed on                       8 June 1998 (08.06.98) US                               60/093,068 (CON) Filed on                       16 July 1998 (16.07.98) US                               60/113,864 (CON) Filed on                       24 December 1998 (24.12.98)  <b>(71) Applicant (for all designated States except US):</b> ADVANCED MEDICINE, INC. [US/US]; 280 Utah Avenue, South San Francisco, CA 94080 (US).	<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> JACOBSEN, John, R. [US/US]; 23 Cityview Way, San Francisco, CA 94131 (US). EASTMAN, Donna [US/US]; 37 Don Gabriel Way, Orinda, CA 94563 (US). GRIFFIN, John, H. [US/US]; 56 Walnut Avenue, Atherton, CA 94027 (US).  <b>(74) Agents:</b> SWISS, Gerald, F. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).  <b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title: NOVEL POTASSIUM CHANNEL DRUGS AND THEIR USES</b>		
<b>(57) Abstract</b>		
<p>This invention relates to novel multibinding compounds that bind to potassium (K<sup>+</sup>) channels and modulate their activity. The compounds of this invention comprise 2-10 K<sup>+</sup> channel ligands covalently connected by a linker or linkers, wherein the ligands in their monovalent (i.e., unlinked) state bind to one or more types of K<sup>+</sup> channel. The manner of linking the ligands together is such that the multibinding agents thus formed demonstrate an increased biologic and/or therapeutic effect as compared to the same number of unlinked ligands made available for binding to the K<sup>+</sup> channel. The invention also relates to methods of using such compounds and to methods of preparing them. The compounds of this invention are particularly useful for treating diseases and conditions of mammals that are mediated by K<sup>+</sup> channels. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.</p>		

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<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 38/00, 39/00, 39/44, 39/395, 51/00, C07K 2/00, 4/00, G01N 33/53, 33/543, 33/566</b>		<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/64050</b> <b>(43) International Publication Date:</b> 16 December 1999 (16.12.99)
<b>(21) International Application Number:</b> PCT/US99/12777 <b>(22) International Filing Date:</b> 7 June 1999 (07.06.99)  <b>(30) Priority Data:</b> 60/088,465 8 June 1998 (08.06.98) US 60/093,068 16 July 1998 (16.07.98) US 60/113,864 24 December 1998 (24.12.98) US  <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications</b> US 60/088,465 (CON) Filed on 8 June 1998 (08.06.98) US 60/093,068 (CON) Filed on 16 July 1998 (16.07.98) US 60/113,864 (CON) Filed on 24 December 1998 (24.12.98)  <b>(71) Applicant (for all designated States except US):</b> ADVANCED MEDICINE, INC. [US/US]; 280 Utah Avenue, South San Francisco, CA 94080 (US).		<b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> JACOBSEN, John, R. [US/US]; 23 Cityview Way, San Francisco, CA 94131 (US). EASTMAN, Donna [US/US]; 37 Don Gabriel Way, Orinda, CA 94563 (US). GRIFFIN, John, H. [US/US]; 56 Walnut Avenue, Atherton, CA 94027 (US).  <b>(74) Agents:</b> SWISS, Gerald, F. et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).  <b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
<b>(54) Title:</b> NOVEL POTASSIUM CHANNEL DRUGS AND THEIR USES			
<b>(57) Abstract</b> <p>This invention relates to novel multibinding compounds that bind to potassium (K<sup>+</sup>) channels and modulate their activity. The compounds of this invention comprise 2-10 K<sup>+</sup> channel ligands covalently connected by a linker or linkers, wherein the ligands in their monovalent (i.e., unlinked) state bind to one or more types of K<sup>+</sup> channel. The manner of linking the ligands together is such that the multibinding agents thus formed demonstrate an increased biologic and/or therapeutic effect as compared to the same number of unlinked ligands made available for binding to the K<sup>+</sup> channel. The invention also relates to methods of using such compounds and to methods of preparing them. The compounds of this invention are particularly useful for treating diseases and conditions of mammals that are mediated by K<sup>+</sup> channels. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.</p>			

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## NOVEL POTASSIUM CHANNEL DRUGS AND THEIR USES

### CROSS-REFERENCE TO RELATED APPLICATIONS

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This application claims priority to U.S. Applications Serial Nos. 60/088,465, filed June 8, 1998; 60/093,068, filed July 16, 1998; and 60/113,864, filed December 24, 1998, the entire contents of which are incorporated herein by reference.

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### BACKGROUND

#### Field of the Invention

This invention relates to novel multibinding compounds that bind to potassium ( $K^+$ ) channels and modulate their activity. The compounds of this invention comprise 2-10  $K^+$  channel ligands covalently connected by a linker or linkers, wherein the ligands in their monovalent (i.e., unlinked) state bind to one or more types of  $K^+$  channel. The manner of linking the ligands together is such that the multibinding agents thus formed demonstrate an increased biologic and/or therapeutic effect as compared to the same number of unlinked ligands made available for binding to the  $K^+$  channel. The invention also relates to methods of using such compounds and to methods of preparing them.

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The compounds of this invention are particularly useful for treating diseases and conditions of mammals that are mediated by  $K^+$  channels. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

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15       The disclosure of each of the above publications is incorporated herein by reference in its entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety.

#### State of the Art

20       Voltage-regulated potassium channels mediate the flux of K<sup>+</sup> out of cells in response to changes in membrane potential.<sup>28</sup> Voltage-gated K<sup>+</sup> channels in the open state typically transition to an inactivated state, and must reacquire the ability to respond to an external stimulus during a recovery period. An inward rectifying voltage-regulated potassium channel in cardiac muscle is also activated by acetylcholine (i.e., it is gated by more than one type of stimulus).<sup>18</sup> A calcium-activated K<sup>+</sup> channel has been described.<sup>16</sup> Potassium channels serve  
25       a variety of important cellular functions, including excitability, setting and maintaining the resting potential, repolarizing action potentials, transmembrane transport, volume regulation, signal transduction, and so on.<sup>28</sup> They are implicated in a variety of pathophysiological disorders, including hypertension, cardiac arrhythmogenesis, insulin-dependent diabetes, non-

insulin dependent diabetes mellitus, diabetic neuropathy, seizures, tachycardia, ischemic heart disease, cardiac failure, angina, myocardial infarction, transplant rejection, autoimmune disease, sickle cell anemia, muscular dystrophy, gastrointestinal disease, mental disorder, sleep disorder, anxiety disorder, neurosis, alcoholism, inflammation, cerebrovascular ischemia, CNS diseases, epilepsy, Parkinson's disease, asthma, incontinence, urinary dysfunction, micturition disorder, irritable bowel syndrome, restenosis, subarachnoid hemorrhage, Alzheimers disease, and they mediate the transmission of pain impulses by peripheral nerves.<sup>45</sup>

Figure 1 illustrates in cross-sectional view the transmembrane domain/subunit organization of various transporter molecules, as it is presently understood by those working in the field of transport physiology. It should be understood that, for purposes of simplification, other subunits that may be involved in or required for transporter activity have been omitted from the diagram.

Referring to Figure 1, voltage-gated ion channels and related proteins are tetrameric structures formed by the noncovalent association of individual subunits (1),(2), or by the interaction of homologous domains of a monomeric protein (3). The channels differ as well in the number of transmembrane segments per subunit or per domain. Inward-rectifier type K<sup>+</sup> channels and P<sub>2x</sub> purinergic channels have two transmembrane-segments in each subunit, Shaker-type K<sup>+</sup> channels have six transmembrane segments per subunit and Na<sup>+</sup> and Ca<sup>++</sup> channels have six transmembrane segments per domain. Neurotransmitter-gated ion channels such as those shown in (4) are organized as pentamers, with each of the subunits having four transmembrane segments/domains. The activation gate for potassium channels has not been identified, although a trap door mechanism has been proposed.<sup>81,120</sup>

Potassium channels are structurally similar to, but smaller and simpler than, sodium and calcium ion channels,<sup>98</sup> with the K<sup>+</sup> channel tetrameric structure being formed by four polypeptides.<sup>3</sup> However, potassium channels represent a diverse class of ion channels.<sup>18</sup>

Homotetramers can form, but there is evidence that heterotetramers may be functionally relevant *in vivo*.<sup>10</sup> The x-ray structure of a bacterial K<sup>+</sup> channel (which is homologous to mammalian K<sup>+</sup> channels) has been disclosed.<sup>21</sup> A prokaryotic K<sup>+</sup> channel was found to have the same structure as a eukaryotic K<sup>+</sup> channel.<sup>104</sup> The channel has an inverted teepee structure with a large hydrophobic cavity. The cavity (10Å) is centered in the channel on the cytoplasmic side, and appears to get larger upon channel opening.<sup>21,82,110,114</sup> Voltage-dependent cardiac potassium channel genes have been cloned as cDNAs.<sup>10,113,116</sup> Variability in the potassium channel genes may relate to disease conditions.<sup>14,48,50,70</sup>

Drug binding sites for tetraethylammonium, quinidine and 4-aminopyridine are found in the inner vestibule of the K<sup>+</sup> channel, and the amino acid side chains involved are localized in the S6 helix. Binding studies using mutagenesis show similarity to local anesthetic (LA) binding to sodium channels, although sodium channel inhibitors bind more deeply in the cavity.<sup>72,88</sup>

There appear to be two types of potassium channel inactivation, N-type and C-type, which can occur simultaneously in Shaker potassium channels. Both are partially coupled to activation and are usually voltage insensitive once activation is complete. N-type inactivation in Shaker B channels depends on a group of amino acids at the N-terminal that bind to the activated channel and occlude the intracellular mouth of the channel. No sequence similarity has been found among the N-termini of the N-type inactivating channels. N-type inactivation is voltage insensitive at positive potentials and competes with drug binding at the intracellular face of the channel. C-type inactivation, which is less understood, occurs by occlusion of the external mouth of the channel during sustained depolarization. C-type inactivation is voltage insensitive at potentials where activation is complete, but recovery from C-type inactivation is voltage sensitive. Both C- and N-type inactivation are coupled or partially coupled to activation, and both require similar degrees of activation to proceed.<sup>40</sup>

Not surprisingly, potassium channels are recognized as important targets for drug therapy. For example, potassium channels are targeted by certain antidiabetic, antihypertensive and antiarrhythmic drugs.

5 Potassium channel antagonists are used for treatment of arrhythmia. Antiarrhythmic agents are classified into four classes under the Vaughan Williams classification scheme: Class I (sodium channel blockers); Class II (beta-blockers); Class III (potassium channel blockers); and Class IV (calcium channel blockers). As shown in Table 1, an antiarrhythmic agent may have activity in several channels and/or with several receptors.<sup>89,92,101</sup> Newer drugs  
10 are more selective to specific K<sup>+</sup> channels, as shown in Table 2. Properties of some known K<sup>+</sup> channel blockers are given in Table 3. Table 5 sets forth the principal K<sup>+</sup> currents and some drugs that block them.<sup>45</sup> The majority of drugs in development are I<sub>Kr</sub> blockers.<sup>87,103,112</sup> Some agents appear to be cationic open-channel blockers.<sup>115,118,119</sup>

15 Combination therapy with two separate agents, e.g., a potassium channel opener with little or no effect on cardiac action potential and a Class III antiarrhythmic compound has been disclosed.<sup>75</sup>

The clinical shortcomings of drugs in current usage are considerable. Their most  
20 common adverse side effects include headache, hypotension, nausea, vomiting, dizziness, and the like. Other side effects may include photo sensitivity, corneal microdeposits, neuropathy, fatigue,<sup>35,39,41,56,61</sup> pneumonitis, hepatotoxicity, proarrhythmic effects,<sup>46</sup> thyroid abnormalities and bradycardia. Reverse use dependence, which may lead to torsades de pointes (an induced arrhythmia), is a major problem of most or all known Class III agents.<sup>42,43,47,96,117</sup> Further,  
25 there may be no survival benefit associated with the use of these agents.<sup>67</sup> With few exceptions, the currently used drugs have a short duration of action and must be administered frequently for sustained effects.

Thus, there continues to exist a need for novel compounds with greater tissue selectivity, increased efficacy, reduced side effects and a more favorable duration of action.

## SUMMARY OF THE INVENTION

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This invention is directed to novel multibinding compounds that bind to K<sup>+</sup> channels in mammalian tissues and can be used to treat diseases and conditions mediated by such channels.

10

This invention is also directed to general synthetic methods for generating large libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties for potassium channels. The diverse multimeric compound libraries provided by this invention are synthesized by combining a linker or linkers with a ligand or ligands to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity and polarization. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

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This invention is also directed to libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties. These libraries are prepared via the methods described above and permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a ligand or a class of ligands targeting a potassium channel.

Accordingly, in one of its composition aspects, this invention is directed to a multibinding compound and salts thereof comprising 2 to 10 ligands which may be the same

or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel.

The multibinding compounds of this invention are preferably represented by Formula

5 I:



where each L is a ligand that may be the same or different at each occurrence; X is a linker that may be the same or different at each occurrence; *p* is an integer of from 2 to 10; and *q* is an integer of from 1 to 20; wherein each of said ligands comprises a ligand domain  
10 capable of binding to a K<sup>+</sup> channel. Preferably *q* is less than *p*.

Preferably, the binding of the multibinding compound to a K<sup>+</sup> channel or channels in a mammal modulates diseases and conditions mediated by the K<sup>+</sup> channel or channels.

15 In another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of one or more multibinding compounds (or pharmaceutically acceptable salts thereof) comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said  
20 ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel of a cell mediating mammalian diseases or conditions, thereby modulating the diseases or conditions.

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a  
25 therapeutically effective amount of one or more multibinding compounds represented by Formula I:



or pharmaceutically acceptable salts thereof, where each L is a ligand that may be the same or different at each occurrence; X is a linker that may be the same or different at each

occurrence;  $p$  is an integer of from 2 to 10; and  $q$  is an integer of from 1 to 20; wherein each of said ligands comprises a ligand domain capable of binding to a  $K^+$  channel of a cell mediating mammalian diseases or conditions, thereby modulating the diseases or conditions. Preferably  $q$  is less than  $p$ .

5

In one of its method aspects, this invention is directed to a method for modulating the activity of a  $K^+$  channel in a biologic tissue, which method comprises contacting a tissue having a  $K^+$  channel with a multibinding compound (or pharmaceutically acceptable salts thereof) under conditions sufficient to produce a change in the activity of the channel in said tissue, wherein the multibinding compound comprises 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a  $K^+$  channel.

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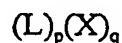
In another of its method aspects, this invention is directed to a method for treating a disease or condition in a mammal resulting from an activity of a  $K^+$  channel, which method comprises administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds (or pharmaceutically acceptable salts thereof) comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a  $K^+$  channel of a cell mediating mammalian diseases or conditions.

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In yet another of its method aspects, this invention is directed to a method for treating a disease or condition in a mammal resulting from an activity of a  $K^+$  channel, which method comprises administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds represented by Formula I:

25



I



and pharmaceutically acceptable salts thereof, where each L is a ligand that may be the same or different at each occurrence; X is a linker that may be the same or different at each occurrence;  $p$  is an integer of from 2 to 10; and  $q$  is an integer of from 1 to 20; wherein each of said ligands comprises a ligand domain capable of binding to a  $K^+$  channel of a cell  
5 mediating mammalian diseases or conditions. Preferably  $q$  is less than  $p$ .

In a further aspect, this invention provides processes for preparing the multibinding agents of Formula I. This can be accomplished by combining  $p$  appropriately functionalized ligands with  $q$  complementary functionalized linkers under conditions where covalent bond  
10 formulation between the ligands and linkers occurs; alternatively, linking portions of  $p$  appropriately functionalized ligands to  $q$  complementary functionalized linkers and then completing the synthesis of the ligands in a subsequent step may be performed to prepare these compounds. Another method which may be used involves linking  $p$  appropriately functionalized ligands to portions of the linker(s) and then completing the synthesis of the  
15 linker(s) in a subsequent step. Coupling one or more of an appropriately functionalized ligand to a complementary functionalized linker, and subsequently coupling one or more additional ligands to said linker or linkers may be done to prepare the claimed compounds. Coupling as above wherein coupling of different appropriately functionalized linkers occurs simultaneously may also be used.

20

In one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

- (a) identifying a ligand or a mixture of ligands wherein each ligand contains at  
25 least one reactive functionality;
- (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;  
5 and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

In another of its method aspects, this invention is directed to a method  
10 for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at  
15 least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional  
20 groups react to form a covalent linkage between said linker and at least two of said ligands;  
and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

25 The preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b). Sequential addition is preferred when a mixture of different ligands is employed to ensure heterodimeric or multimeric

compounds are prepared. Concurrent addition of the ligands occurs when at least a portion of the multimer compounds prepared are homomultimeric compounds.

5 The assay protocols recited in (d) can be conducted on the multimeric ligand compound library produced in (c) above, or preferably, each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

10 In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties for potassium channels, which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

15 (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

20

In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties for potassium channels, which library is prepared by the method comprising:

25 (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

5

In a preferred embodiment, the library of linkers employed in either the methods or the library aspects of this invention is selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and amphiphilic linkers. For example, in one embodiment, each of the linkers in the linker library may comprise linkers of different chain length and/or having different complementary reactive groups. Such linker lengths can preferably range from about 2 to 100Å.

10

In another preferred embodiment, the potassium channel ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands in order to provide for a range of orientations of said ligand on said multimeric ligand compounds. Such reactive functionality includes, by way of example, carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, and precursors thereof. It is understood, of course, that the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

15

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In other embodiments, the multimeric ligand compound is homomeric (i.e., each of the ligands is the same, although it may be attached at different points) or heterodimeric (i.e., at least one of the ligands is different from the other ligands).

25

In addition to the combinatorial methods described herein, this invention provides for an iterative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor. Specifically, this method aspect is directed to a method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

Preferably, steps (e) and (f) are repeated at least two times, more preferably at from 2-50 times, even more preferably from 3 to 50 times, and still more preferably at least 5-50 times.

5

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a highly schematic illustration of the transmembrane organization of various cell membrane transporters.

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Figure 2 illustrates a method for optimizing the linker geometry for presentation of ligands (filled circles) in bivalent compounds:

- A. phenyldiacetylene core structure
- B. cyclohexane dicarboxylic acid core structure

15

Figure 3 shows exemplary linker "core" structures.

Figure 4 illustrates examples of multi-binding compounds comprising (A) 2 ligands, (B) 3 ligands, (C) 4 ligands, and (D) >4 ligands attached in different formats to a linker.

20

Figure 5 illustrates the ligand amiodarone, which may be used in preparing multi-binding compounds. Potentially modifiable positions are indicated by arrows.

Figure 6 illustrates numerous reactive functional groups and the resulting bonds formed by reaction therebetween.

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Figures 7 to 21 illustrate convenient methods for preparing the multibinding compounds of this invention. In each of these figures, the filled circles represent linkers, referred to in the written Examples as "Link".

## DETAILED DESCRIPTION OF THE INVENTION

Biological systems in general are controlled by molecular interactions between bioactive ligands and their receptors, in which the receptor "recognizes" a molecule or a portion thereof (i.e., a ligand domain) to produce a biological effect. The K<sup>+</sup> channels are considered to be pharmacological receptors: they possess specific binding sites for ligands having agonist and antagonist activities; the binding of ligands to such sites modulates K<sup>+</sup> flux through the channel; the channel properties (i.e., gating and ion selectivity) are regulatable. Accordingly, diseases or conditions that involve, or are mediated by, K<sup>+</sup> channels can be treated with pharmacologically active ligands that interact with such channels to initiate, modulate or abrogate transporter activity.

The interaction of a K<sup>+</sup> channel and a K<sup>+</sup> channel-binding ligand may be described in terms of "affinity" and "specificity". The "affinity" and "specificity" of any given ligand-K<sup>+</sup> channel interaction is dependent upon the complementarity of molecular binding surfaces and the energetic costs of complexation (i.e., the net difference in free energy between bound and free states). Affinity may be quantified by the equilibrium constant of complex formation, the ratio of on/off rate constants, and/or by the free energy of complex formation. Specificity relates to the difference in binding affinity of a ligand for different receptors.

The net free energy of interaction of such ligand with a K<sup>+</sup> channel is the difference between energetic gains (enthalpy gained through molecular complementarity and entropy gained through the hydrophobic effect) and energetic costs (enthalpy lost through decreased solvation and entropy lost through reduced translational, rotational and conformational degrees of freedom).

The compounds of this invention comprise 2 to 10 K<sup>+</sup> channel-binding ligands covalently linked together and capable of acting as multibinding agents. Without wishing to be bound by theory, the enhanced activity of these compounds is believed to arise at least in

part from their ability to bind in a multivalent manner with multiple ligand binding sites on a K<sup>+</sup> channel or channels, which gives rise to a more favorable net free energy of binding.

Multivalent interactions differ from collections of individual monovalent (univalent) interactions by being capable of providing enhanced biologic and/or therapeutic effect.

5 Multivalent binding can amplify binding affinities and differences in binding affinities, resulting in enhanced binding specificity as well as affinity.

### Definitions

As used herein:

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The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, preferably 1-10 carbon atoms, more preferably 1-6 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, secondary butyl, tert-butyl, n-hexyl, n-octyl, n-decyl, n-dodecyl, 2-ethyldodecyl, 15 tetradecyl, and the like, unless otherwise indicated.

15

The term "substituted alkyl" refers to an alkyl group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, 20 amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and -NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or 25 different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

25

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, preferably 1-10 carbon



atoms, more preferably 1-6 carbon atoms. This term is exemplified by groups such as methylene ( $-\text{CH}_2-$ ), ethylene ( $-\text{CH}_2\text{CH}_2-$ ), the propylene isomers (e.g.,  $-\text{CH}_2\text{CH}_2\text{CH}_2-$  and  $-\text{CH}(\text{CH}_3)\text{CH}_2-$ ) and the like.

5           The term "substituted alkylene" refers to: (1) An alkylene group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, 10   aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocyclooxy, thioheterocyclooxy, nitro, and  $-\text{NR}_a\text{R}_b$ , wherein  $\text{R}_a$  and  $\text{R}_b$  may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form 15   one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group; (2) An alkylene group as defined above that is interrupted by 1-20 atoms independently chosen from oxygen, sulfur and  $\text{NR}_a-$ , where  $\text{R}_a$  is chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic, or groups 20   selected from carbonyl, carboxyester, carboxyamide and sulfonyl; and (3) An alkylene group as defined above that has both from 1 to 5 substituents as defined above and is also interrupted by 1-20 atoms as defined above. Examples of substituted alkenes are chloromethylene ( $-\text{CH}(\text{Cl})-$ ), aminoethylene ( $-\text{CH}(\text{NH}_2)\text{CH}_2-$ ), 2-carboxypropylene isomers ( $-\text{CH}_2\text{CH}(\text{CO}_2\text{H})\text{CH}_2-$ ), ethoxyethyl ( $-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-$ ), ethylmethylaninoethyl 25   ( $-\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_2-$ ), 1-ethoxy-2-(2-ethoxy-ethoxy)ethane ( $-\text{CH}_2\text{CH}_2\text{O}-\text{CH}_2\text{CH}_2-\text{OCH}_2\text{CH}_2-\text{OCH}_2\text{CH}_2-$ ), and the like.

The term "alkaryl" or "aralkyl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl in which alkylene and aryl are as defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

5 The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

10 The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

15 The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Examples of such groups are methylenemethoxy ( $-\text{CH}_2\text{OCH}_3$ ), ethylenemethoxy ( $-\text{CH}_2\text{CH}_2\text{OCH}_3$ ), n-propylene-iso-propoxy ( $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OCH}(\text{CH}_3)_2$ ), methylene-t-butoxy ( $-\text{CH}_2-\text{O}-\text{C}(\text{CH}_3)_3$ ) and the like.

20 The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein.

25 Preferred alkylthioalkoxy groups are alkylene-S-alkyl and include, by way of example, methylenethiomethoxy ( $-\text{CH}_2\text{SCH}_3$ ), ethylenethiomethoxy ( $-\text{CH}_2\text{CH}_2\text{SCH}_3$ ), n-propylene-iso-thiopropoxy ( $-\text{CH}_2\text{CH}_2\text{CH}_2\text{SCH}(\text{CH}_3)_2$ ), methylene-t-thiobutoxy ( $-\text{CH}_2\text{SC}(\text{CH}_3)_3$ ) and the like.

"Alkenyl" refers to a monoradical of a branched or unbranched unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 double bonds. This term is further exemplified by such radicals as vinyl, prop-2-enyl, pent-3-enyl, hex-5-enyl, 5-ethyldodec-3,6-dienyl, and the like.

The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, aryloxy, thioaryloxy, heteroaryloxy, thioheteroaryloxy, heterocyclooxy, thioheterocyclooxy, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and, -NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

"Alkenylene" refers to a diradical of an unsaturated hydrocarbon, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 double bonds. This term is further exemplified by such radicals as 1,2-ethenyl, 1,3-prop-2-enyl, 1,5-pent-3-enyl, 1,4-hex-5-enyl, 5-ethyl-1,12-dodec-3,6-dienyl, and the like.

The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocyclooxy, thioheterocyclooxy, nitro, and NR<sup>a</sup>R<sup>b</sup>,

wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

"Alkynyl" refers to a monoradical of an unsaturated hydrocarbon, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 triple bonds. This term is further exemplified by such radicals as acetylenyl, prop-2-ynyl, pent-3-ynyl, hex-5-ynyl, 5-ethyldodec-3,6-diynyl, and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyacylamino, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, thioaryloxy, heteroaryl, heteroaryloxy, thioheteroaryloxy, heterocyclic, heterocycloxy, thioheterocycloxy, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, SO<sub>2</sub>-heterocyclic, NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

"Alkynylene" refers to a diradical of an unsaturated hydrocarbon radical, preferably having from 2 to 40 carbon atoms, preferably 2-10 carbon atoms, more preferably 2-6 carbon atoms, and preferably having 1-6 triple bonds. This term is further exemplified by such radicals as 1,3-prop-2-ynyl, 1,5-pent-3-ynyl, 1,4-hex-5-ynyl, 5-ethyl-1,12-dodec-3,6-diynyl, and the like.

5 The term "acyl" refers to the groups -CHO, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

10 The term "acylamino" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholine) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

15 The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyloxy" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

20 The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

25 The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl).

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, trihalomethyl, NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "arylene" refers to a diradical derived from aryl or substituted aryl as defined above, and is exemplified by 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "amino" refers to the group -NH<sub>2</sub>.

The term "substituted amino" refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" refers to the group "-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl", "-C(O)O-alkynyl" and "-C(O)O-substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl where alkynyl are as defined herein.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring or fused rings and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl,

substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and NR<sup>a</sup>R<sup>b</sup>, wherein R<sup>a</sup> and R<sup>b</sup> may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

"Haloalkyl" refers to alkyl as defined above substituted by 1-4 halo groups as defined above, which may be the same or different, such as 3-fluorododecyl, 12,12,12-trifluorododecyl, 2-bromooctyl, -3-bromo-6-chloroheptyl, and the like.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, trihalomethyl,



mono- and di-alkylamino, mono- and  $\text{NR}^a\text{R}^b$ , wherein  $\text{R}^a$  and  $\text{R}^b$  may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

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The term "heteroaryloxy" refers to the group heteroaryl-O-.

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The term "heteroarylene" refers to the diradical group derived from heteroaryl or substituted heteroaryl as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylenes, 1,8-quinolinylenes, 1,4-benzofuranylene, 2,5-pyridinylenes, 1,3-morpholinylenes, 2,5-indolenyl, and the like.

15

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

20

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Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO<sub>2</sub>-alkyl, -SO<sub>2</sub>-substituted alkyl, -SO<sub>2</sub>-aryl, -SO<sub>2</sub>-heteroaryl, and  $\text{NR}^a\text{R}^b$ , wherein  $\text{R}^a$  and  $\text{R}^b$  may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic. Such heterocyclic groups can have a single ring or multiple condensed rings.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidiny, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

A preferred class of heterocyclics include "crown compounds" which refers to a specific class of heterocyclic compounds having one or more repeating units of the formula  $[-(\text{CH}_2)_m\text{Y}-]$  where  $m$  is equal to or greater than 2, and  $\text{Y}$  at each separate occurrence can be O, N, S or P. Examples of crown compounds include, by way of example only,  $[-(\text{CH}_2)_3\text{NH}-]_3$ ,  $[-((\text{CH}_2)_2\text{O})_4-((\text{CH}_2)_2\text{NH})_2]$  and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term "heterocyclooxy" refers to the group heterocyclic-O-.

The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

The term "heterocyclene" refers to the diradical group derived from a heterocycle as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

The term "oxyacylamino" refers to the group  $-\text{OC}(\text{O})\text{NRR}$  where each  $\text{R}$  is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "thiol" refers to the group -SH.

The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

5       The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

10       The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

15       As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

20       "Alkyl optionally interrupted by 1-5 atoms chosen from O, S, or N" refers to alkyl as defined above in which the carbon chain is interrupted by O, S, or N. Within the scope are ethers, sulfides, and amines, for example 1-methoxydecyl, 1-pentyloxynonane, 1-(2-isopropoxyethoxy)-4-methylnonane, 1-(2-ethoxyethoxy)dodecyl, 2-(t-butoxy)heptyl, 1-pentylsulfanylnonane, nonylpentylamine, and the like.

25       "Heteroarylalkyl" refers to heteroaryl as defined above linked to alkyl as defined above, for example pyrid-2-ylmethyl, 8-quinolinylpropyl, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, optionally

substituted alkyl means that alkyl may or may not be substituted by those groups enumerated in the definition of substituted alkyl.

5 The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the multibinding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multibinding compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

10 Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl)  
15 amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines,  
20 disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl,  
25 substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group. See, generally, T.W. Greene & P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 2<sup>nd</sup> Ed., 1991, John Wiley and Sons, N.Y.

The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidene, phenacyl, *t*-butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively

removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

Preferred removable amino blocking groups include conventional substituents such as  
5 t-butyloxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), fluorenylmethoxycarbonyl (Fmoc), allyloxycarbonyl (ALOC) and the like, which can be removed by conventional conditions compatible with the nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl,  
10 t-butyl etc. which can be removed by mild hydrolysis conditions compatible with the nature of the product.

As used herein, the terms "inert organic solvent" or "inert solvent" mean a solvent  
inert under the conditions of the reaction being described in conjunction therewith [including,  
15 for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform ("CHCl<sub>3</sub>"), methylene chloride (or dichloromethane or "CH<sub>2</sub>Cl<sub>2</sub>"), diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol, isopropanol, tert-butanol, dioxane, pyridine, and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert solvents.

20 The term "K<sup>+</sup> channel" refers to a structure comprised of integral membrane proteins that functions to allow K<sup>+</sup> to equilibrate across a membrane according to its electrochemical gradient and at rates that are diffusion limited.

25 "Ligand" as used herein denotes a compound that is a binding partner for a K<sup>+</sup> channel receptor, and is bound thereto, for example, by complementarity. The specific region or regions of the ligand molecule that is recognized by the ligand binding site of a K<sup>+</sup> channel receptor is designated as the "ligand domain". A ligand may be either capable of binding to a

receptor by itself, or may require the presence of one or more non-ligand components for binding (e.g. ions, a lipid molecule, a solvent molecule, and the like).

Ligands useful in this invention comprise K<sup>+</sup> channel modulators such as quinidine,<sup>6,94</sup>  
5 glibenclamide, procaine, tetraethyl ammonium,<sup>20</sup> clofilium,<sup>102</sup> melperone,<sup>8</sup> pinacidil, WAY-  
123,398,<sup>91</sup> cromakalim,<sup>26</sup> propofol, thiopentone,<sup>32</sup> risotilide, almokalant,<sup>36</sup> bretylium,<sup>38</sup> N-  
acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356,<sup>45</sup> afinidine,<sup>49</sup>  
chromanol 293B,<sup>57</sup> L-768,673 and its analogs,<sup>53</sup> bethanidine,<sup>54</sup> disopyramide,<sup>23</sup>  
desethylamiodarone,<sup>1</sup> NE-10064,<sup>9,84</sup> artilide,<sup>11</sup> dofetilide,<sup>19,73,74,90,99</sup> E-4031,<sup>24,99,109</sup>  
10 sematilide,<sup>106,107</sup> ambasilide, azimilide,<sup>5,80,86,93,100,108</sup> tedisamil, dronedarone,<sup>79</sup> ibutilide,<sup>78,111</sup>  
sotalol,<sup>83</sup> benzodiazepine analogs<sup>76,77</sup> and amiodarone.<sup>55,65,83,95,105</sup> See Table 4 for structures of  
various potassium channel ligands.

While it is contemplated that many potassium channel ligands that are currently  
15 known can be used in the preparation of multibinding compounds of this invention (Table 2),  
it should be understood that portions of the ligand structure that are not essential for  
molecular recognition and binding activity (i.e., that are not part of the ligand domain) may be  
varied substantially, replaced with unrelated structures and, in some cases, omitted entirely  
without affecting the binding interaction. Accordingly, it should be understood that the term  
20 "ligand" is not intended to be limited to compounds known to be useful as K<sup>+</sup> channel  
receptor-binding compounds (e.g., known drugs), in that ligands that exhibit marginal activity  
or lack useful activity as monomers can be highly active as multibinding compounds, because  
of the biological benefit conferred by multivalency. The primary requirement for a ligand as  
defined herein is that it has a ligand domain, as defined above, which is available for binding  
25 to a recognition site on a K<sup>+</sup> channel.

For purposes of the present invention, the term "ligand" or "ligands" is intended to  
include the racemic ligands as well as the individual stereoisomers of the ligands, including  
pure enantiomers and non-racemic mixtures thereof. The scope of the invention as described

and claimed encompasses the racemic forms of the ligands as well as the individual enantiomers and non-racemic mixtures thereof.

5 The term "ligand binding site" as used herein denotes a site on a  $K^+$  channel receptor that recognizes a ligand domain and provides a binding partner for the ligand. The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example agonism, antagonism, modulation, or may maintain an ongoing biological event, and the like.

10 It should be recognized that the ligand binding sites of  $K^+$  channel receptors that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and intermolecular associations. For example,  $K^+$  channel ligand binding sites may be covalently joined in a single structure, noncovalently associated in one or more multimeric structures, embedded in a membrane or biopolymer matrix, and so on, and  
15 therefore have less translational and rotational freedom than if the same sites were present as monomers in solution.

The terms "agonism" and "antagonism" are well known in the art. As used herein, the term "agonist" refers to a ligand that when bound to a  $K^+$  channel stimulates its activity. The  
20 term "antagonist" refers to a ligand that when bound to a  $K^+$  channel inhibits its activity. Channel block or activation may result from allosteric effects of ligand binding to the channel rather than occupancy of the channel pore. These allosteric effects may produce changes in protein conformation that affect  $K^+$  binding sites, gating mechanisms and/or the pore region (i.e., ion permeation).

25 A potassium channel can exist in several modes: C (closed resting state);  $C^*$  (activated closed state); O (open state); and I (inactivated state).<sup>44</sup> The probability that a channel will exist in one of these four states changes with voltage. A given ligand may have



of aqueous potassium sodium tartrate. The organic phase is separated, dried and evaporated, and the residue is chromatographed to afford N-ethyl 4-(4-methylsulfonylamino-phenyl)-4-hydroxybutylamine, **9B-1**.

5           D.     The compound **9B-1** (1mmol), diisopropylethylamine (2 mmol) and 1,6-dibromohexane (0.5 mmol) are dissolved in MeCN (25 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute Na<sub>2</sub>CO<sub>3</sub>, and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated, and the residue is chromatographed to afford the title compound **9B-2**, in which Link is (CH<sub>2</sub>)<sub>6</sub>.

10           E.     In a similar manner, by employing different dialkylating agents, as described herein, in place of 1,6-dibromohexane, different compounds of Formula **9B-2** are obtained.

#### Example 9. (Figure 10A)

15     Preparation of 1,8-di-[4-[2-[diethylaminoethyl]aminocarbonyl]phenylamino-sulfonyl]octane, **10A-2**, in which Link is (CH<sub>2</sub>)<sub>6</sub>.

20           A.     Procaine amide (**10A-1**) (10 mmol) is dissolved in MeCN (50 mL) and 1,8-di-(chlorosulfonyl)octane (5 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute Na<sub>2</sub>CO<sub>3</sub> and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated to afford the title compound, **10A-2**, in which Link is (CH<sub>2</sub>)<sub>6</sub>.

25           B.     In a similar manner, by employing different disulfonyl chlorides, as described herein, different compounds of Formula **10A-2** are obtained.

#### Example 10. (Figure 10B)

Preparation of 1,8-di-[N-ethyl N'-[2-[4-[methylsulfonylamino]benzoylaminoethyl]-amino] 3,5-dioxaoctane, **10B-2**, in which Link is (CH<sub>2</sub>)<sub>2</sub>(O(CH<sub>2</sub>)<sub>2</sub>)<sub>2</sub>.

A. N-Ethyl N'-(4-methylsulfonylaminobenzoyl)ethylenediamine, (10B-1), prepared as described in *J. Med. Chem.*, 1987, 30, 755, (5 mmol) diisopropylethylamine (5 mmol) and 1,8-dibromo-3,5-dioxaoctane (2.5 mmol) are dissolved in MeCN (25 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute Na<sub>2</sub>CO<sub>3</sub> and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The solution is dried and evaporated, and the residue is chromatographed to afford the title compound 10B-2, in which Link is (CH<sub>2</sub>)<sub>2</sub>(O(CH<sub>2</sub>)<sub>2</sub>)<sub>2</sub>.

B. In a similar manner, by employing different dialkylating agents, as described herein, different compounds of Formula 10B-2 are obtained.

#### Example 11. (Figure 11)

Preparation of 1,10-di-[2-hydroxy-2-[4-methylsulfonylaminophenyl]ethylamino]-decane, 11-2, in which Link is (CH<sub>2</sub>)<sub>10</sub>.

A. 1,10-Dibromodecane (5mmol), 2-hydroxy-2-(4-methylsulfonylaminophenyl)-ethylamine (11-1) (10 mmol), prepared as described in European Patent 338793, potassium iodide (0.1g) and K<sub>2</sub>CO<sub>3</sub> (1g) are stirred in MeCN (50mL). The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 11-2, in which Link is (CH<sub>2</sub>)<sub>10</sub>.

B. In a similar manner, by employing different dialkylating agents, as described herein, different compounds of Formula 11-2 are obtained.

#### Example 12. (Figure 12)

Preparation of 1,6-di[4-[1-[[5-(4-chlorophenyl)-2-furanylmethylene]amino]-imidazolidine-2,4-dion-3-yl]butylmethylamino]hexane, 12-2, where Link is (CH<sub>2</sub>)<sub>6</sub>.

A. 1-Benzylamino-3-(4-iodobutyl)imidazolidine-2,4-dione (12-5), prepared as described in W093/04061, (5 mmol) is added to a solution of methylamine (2g) in MeOH (40mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated and the residue is chromatographed to afford 1-benzylamino-3-(4-methylaminobutyl)imidazolin-2,4-dione, 12-6.

B. The latter compound 12-6, (2 mmol) is added to EtOH (25mL) containing 10% Pd/C (50mg) and ammonium formate (0.5g). The progress of the reaction is followed by tlc. When it is complete, the solution is filtered and added to water. The aqueous solution is extracted with EtOAc. The extract is dried and evaporated. The residue is chromatographed to afford 1-amino-3-(4-methylaminobutyl)imidazolidine-2,4-dione, 12-7.

C. The above-described compound (1 mmol) is dissolved in EtOH (20mL). To the solution is added 5-(4-chlorophenyl)furan-2-carboxaldehyde (12-8), (1 mmol) and p-toluenesulfonic acid (10mg). The progress of the reaction is followed by tlc. When it is complete, the mixture is added to water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated to afford 1-[5-(4-chlorophenyl)-2-furanylmethyleneamino]-3-[4-(methylamino)butyl]imidazolidine-2,4-dione, 12-1.

D. A solution of 12-1 (1 mmol), 1,6-di-(p-toluenesulfonyloxy)hexane (0.5 mmol) and diisopropylethylamine (3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) is heated at reflux. The progress of the reaction is monitored by tlc. When it is complete, the solution is cooled and added to water. The aqueous solution is extracted with CH<sub>2</sub>Cl<sub>2</sub>, and the extract is dried and evaporated. The residue is chromatographed to afford the title compound 12-2, in which Link is (CH<sub>2</sub>)<sub>6</sub>.

E. In a similar manner, by employing other dialkylating agents, as described herein, different compounds of Formula 12-2 are obtained.

## Example 13. (Figure 12)

Preparation of 1,4-di[4-[1-[[5-(4-chlorophenyl)-2-furanylmethylene]amino]-imidazolidine-2,4-dione-3-yl]4-butyl(piperazin-1-yl)]butane, 12-4, where Link is (CH<sub>2</sub>)<sub>4</sub>.

5           A.     1-Benzylamino-3-(4-iodobutyl)imidazolidine-2,4-dione (12-5), prepared as described in W093/04061, (5 mmol), diisopropylethylamine (10 mmol) and N-benzyloxycarbonylpiperazine (5 mmol) are dissolved in MeCN (50 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated, and the residue is chromatographed to  
10           afford 1-benzylamino-3-[4-(4-benzyloxycarbonylpiperazinyl)butyl]-imidazoline-2,4-dione, 12-9.

          B.     The above compound, (2 mmol) is dissolved in EtOH (25 mL), and to the solution are added 5% Pd/C (100mg) and ammonium formate (250mg). The progress of the  
15           reaction is monitored by tlc. When it is complete, the mixture is filtered and added to water, then extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford 1-amino-3-[4-(piperazin-1-yl)butyl]imidazoline-2,4-dione, 12-10.

          C.     The above-described compound (1 mmol) is dissolved in EtOH (20mL). To  
20           the solution is added 5-(4-chlorophenyl)furan-2-carboxaldehyde (12-8), (1 mmol) and p-toluenesulfonic acid (10mg). The progress of the reaction is followed by tlc. When it is complete, the mixture is added to water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract is dried and evaporated to afford 1-[5-(4-chlorophenyl)-2-furanylmethyleneamino]-3-[4-(piperazin-1-yl)butyl]imidazolidine-2,4-dione, 12-3.

25           D.     A solution of 1,4-dibromobutane, (0.5 mmol) and 12-3 (1 mmol) in EtOH is maintained at room temperature, while the progress of the reaction is monitored by tlc. When it is complete, the mixture is evaporated to dryness under reduced pressure, and the residue is chromatographed to afford 12-4, in which Link is (CH<sub>2</sub>)<sub>4</sub>.

E. In a similar manner, by employing different dialkylating agents, as described herein, in part D above, different compounds of Formula 12-4 are obtained.

Example 14. (Figure 13)

5 Preparation of 1,10-di-(3-cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo-[3.3.1]non-7-yl)decane, 13-2, in which Link is  $(CH_2)_{10}$ .

A. 3-Benzyl-7-cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, (20-1, in which  $R_1$  is benzyl), the preparation of which is described in European Patent 461574, (Table 1, compound 22), (5 mmol) is dissolved in MeOH (20 mL) containing 5% Pd/C (50mg) and formic acid (0.5 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is filtered and the solvent is removed under reduced pressure. The residue is chromatographed to afford 3-cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, (20-1, in which  $R_1$  is H).  
15 H).

B. 1,10-Dibromodecane (0.5 mmol) is added to a solution of the compound 20-1 in which  $R_1$  is H, prepared as described above, (1 mmol) and diisopropylethylamine (0.5 mL) in DMF (10 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute  $Na_2CO_3$  and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 13-2, in which Link is  $(CH_2)_{10}$ .  
20

C. In a similar manner, by employing different dialkylating agents, as described herein, different compounds of structure 13-2 are obtained.  
25

## Example 15. (Figure 14)

Preparation of 1,4-di-[2-[2-[4-(2-butylbenzofuran-3-yl)carbonyl]-2,6-diiodophenoxyethyl]methylamino]acetylamino]butane, 14-2, in which n is 1 and Link is (CH<sub>2</sub>)<sub>4</sub>.

5

A. The compound 7B-1, prepared according to procedures described in *Eur. J. Med. Chem.*, 1974, 19-25, (5 mmol), diisopropylethylamine (5 mmol) and ethyl bromoacetate (5 mmol) are dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated; the residue is chromatographed to afford ethyl N-methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylaminoacetate, 14-1, in which R is ethyl.

10

B. The above compound (1 mmol) is dissolved in THF (10 mL) and water (3 mL), and lithium hydroxide monohydrate (1.25 mmol) is added. The reaction is monitored by tlc. When it is complete, acetic acid (2 mmol) is added, and the solvents are removed under reduced pressure. The residue is chromatographed to afford N-methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylaminoacetic acid, 14-1, in which R is H.

15

C. The above-prepared compound (1 mmol) is dissolved in DMF (20 mL) and dicyclohexylcarbodiimide (1 mmol) and 1,4-diaminobutane (0.5 mmol) are added. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated; the residue is chromatographed to afford the title compound 14-2, in which n is 1 and Link is (CH<sub>2</sub>)<sub>4</sub>.

20

25

D. In a similar manner, by employing different diamines, as described herein, different compounds of Formula 14-2 are obtained.

## Example 16. (Figure 14)

Preparation of 1,4-di-[3-[4-[2-[4-(methylsulfonylamino)phenoxy]ethyl]-aminoethyl]phenylaminosulfonyl]propylmethylamino]butane, 14-7, in which Link is  $(CH_2)_4$ .

5

A. N-methyl N-(4-aminophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]ethylamine, 14-3, prepared as described in Examples 3A and 3B, (5 mmol) is dissolved in  $CH_2Cl_2$  (25 mL) and 3-azidopropylsulfonylchloride (5 mmol) is added. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated; the residue is chromatographed to afford N-methyl N-[4-(3-azidopropylsulfonyl)aminophenylethyl] 2-[4-(methylsulfonylamino)phenoxy]-ethylamine, 14-5.

10

B. The above-prepared compound (1 mmol) is dissolved in MeOH (20 mL) and 5% Pd/C (50 mg) is added. The mixture is stirred in a hydrogen atmosphere. The progress of the reaction is followed by tlc. When it is complete, the solution is filtered and the solvent is removed under reduced pressure. The residue is redissolved in MeOH (20 mL) and paraformaldehyde (1 mmol) and sodium cyanoborohydride (1 mmol) are added. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford N-methyl N-[4-(3-methylaminopropylsulfonyl)-aminophenylethyl]-2-[4-(methylsulfonylamino)phenoxy]ethylamine, 14-6.

15

20

C. 1,4-Dibromobutane (0.5 mmol) is dissolved in MeCN, and  $K_2CO_3$  (0.5 g) and the compound 14-6 (0.25 mmol) are added. The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 14-7, in which Link is  $(CH_2)_4$ .

25

D. In a similar manner, by employing different dialkylating agents, as described herein, in step C above, different compounds of Formula 14-7 are obtained.

Example 17. (Figure 14)

5 Preparation of 1,8-di-[[N-[2-(4-methylsulfonylaminophenoxy)ethyl] N-2-(4-methylsulfonylaminophenyl)ethyl] 2-aminoethoxy]octane, 14-10, in which Link is  $(\text{CH}_2)_8$ .

10 A. N-2-(4-Methylsulfonylaminophenoxy)ethyl 2-(4-methylsulfonylaminophenyl)-ethylamine, (14-8), the preparation of which is described above in Examples 6A and 6B, (1 mmol) is dissolved in EtOH (20 mL) and to the solution is added 2-bromoethanol (1 mmol) and diisopropylethylamine (5 mmol). The reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford N-(2-hydroxyethyl) N-[2-(4-methylsulfonylaminophenoxy)ethyl] 2-(4-methylsulfonylaminophenyl)ethylamine, 14-9.

20 B. The compound 14-9, (1 mmol) is dissolved in DMSO (10 mL) and KOH (10 mmol) and 1,8-dibromooctane (0.5 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound, 14-10, in which Link is  $(\text{CH}_2)_8$ .

25 C. In a similar manner, by employing different dihalo compounds, in place of 1,8-dibromooctane, different compounds of Formula 14-10 can be obtained.

Example 18. (Figure 15)

Preparation of 1,4,8,12-tetra-[2-(4-methylsulfonylaminophenoxy)ethyl]-1,4,8,12-tetraazacyclohexadecane, 15-3, in which X is O.



A. 2-(4-Methylsulfonylamino)phenoxyethyl bromide (15-1, in which X is O), the preparation of which is described in *J. Med. Chem.*, 1990, 1551, (4 mmol) and 1,4,8,12-tetraazacyclohexadecane (15-2) (1 mmol) are dissolved in MeCN (25 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound, 15-3, in which X is O.

B. In a similar manner, by employing 2-(4-methylsulfonylamino)phenyl)ethylamine, (15-1, in which X is a direct bond, the preparation of which is described in *J. Med. Chem.*, 1990, 1551), there is obtained 1,4,8,12-tetra-[2-(4-methylsulfonylamino)phenyl)ethyl]-1,4,8,12-tetraazacyclohexadecane, 15-3, in which X is a direct bond.

#### Example 19. (Figure 15)

Preparation of 1,3,5-tri-[N-methyl 2-[2,6-diiodo-4-[2-butyl-3-benzofuran-ylcarbonyl]phenoxy]ethyl]aminomethyl]benzene, 15-6.

N-Methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylamine (15-4), prepared according to procedures described in *Eur. J. Med. Chem.*, 1974, 19-25, (3 mmol) is dissolved in MeCN (30 mL), and 1,3,5-tri(bromomethyl)benzene (1 mmol) and  $K_2CO_3$  (0.5g) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 15-6.

#### Example 20. (Figure 15)

Preparation of the trimeric amide 15-9.

A. N-Methyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylamine (15-4), prepared according to procedures described in *Eur. J.*

*Med. Chem.*, 1974, 19-25, (5 mmol) is dissolved in EtOH (25 mL) and ethyl bromoacetate (5 mmol) and diisopropylethylamine (10 mmol) are added. The progress of the reaction is followed by tlc. When it is complete, the reaction is added to water and extracted with EtOAc. The extract is washed with dilute HCl, the dried and the solvent is evaporated under reduced pressure. The residue is dissolved in THF (15 mL), and LiOH, H<sub>2</sub>O (5 mmol) is added. The progress of the reaction is followed by tlc. When it is complete, the pH is adjusted to 7 by addition of dilute HCl. The solvents are removed under reduced pressure and the residue is chromatographed to afford N-methyl N-[2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diodophenoxy]ethyl]glycine, 15-7.

B. The compound 15-7 (3 mmol) is dissolved in DMF (25 mL) and dicyclohexylcarbodiimide (3 mmol) and tris(2-aminoethyl)amine (1 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the triamide product 15-9.

#### Example 21. (Figure 15)

Preparation of 1,4-di-[N-methyl 2-(4-methylsulfonylaminophenoxy)-ethylamino]butane, 15-12, in which X is O, R is methyl and Link is (CH<sub>2</sub>)<sub>4</sub>.

A. 2-(4-Methylsulfonylaminophenoxy)ethyl bromide, 15-10, in which X is O, (2 mmol) and 1,4-di(methylamino)butane, 15-11, (1 mmol) are dissolved in MeCN (20 mL) containing K<sub>2</sub>CO<sub>3</sub> (0.5g). The progress of the reaction is followed by tlc. When it is complete, the reaction is added to water and extracted with EtOAc. The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 15-12.

B. In a similar manner, by employing 2-(4-methylsulfonylaminophenyl)ethyl bromide in A above, there is obtained the corresponding product 1,4-di-[N-methyl 2-(4-

methylsulfonylamino]phenyl)-ethylamino]butane, 15-12, in which X is a direct bond, R is methyl and Link is  $(CH_2)_4$ .

C. In a similar manner, by employing different diamines 15-11, as described herein, in A and B above, there are obtained the corresponding diamine products 15-12.

Example 22. (Figure 18)

Preparation of the asymmetrically linked aminosulfonamide 18-4, in which Link is  $(CH_2)_2$ .

A. N-Methyl N-(4-aminophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]-ethylamine, 18-1, the preparation of which is described in Examples 4A and 4B above, (2 mmol) is dissolved in dry  $CH_2Cl_2$  (25 mL); diisopropylethylamine (10 mmol) and 3-bromopropanesulfonyl chloride (2 mmol) are added. The progress of the reaction is followed by tlc. When it is complete, the reaction is added to water and extracted with EtOAc. The extract is washed and dried and the solvent is evaporated under reduced pressure. The residue is chromatographed to afford 1-bromo-3-[4-[N-methyl 2-[2-[4-methylsulfonylamino]phenoxy]ethylamino]phenylaminosulfonyl]propane, 18-2, in which Link is  $(CH_2)_2$ .

B. N 2-(4-aminophenyl)ethyl 2-[4-methylsulfonylamino]ethylamine, 18-3, prepared using methods described in EP 338793, (1 mmol) and the compound 18-2, (1 mmol) are dissolved in  $CH_2Cl_2$  (25 mL) and to the solution is added diisopropylethylamine (10 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 18-4, in which Link is  $(CH_2)_2$ .

C. In a similar manner, by employing different bromosulfonyl chlorides, as described herein, the corresponding compounds of Formula 18-4 are obtained.

## Example 23. (Figure 19)

**Preparation of the dimeric heterovalomer 19-4, in which Link is (CH<sub>2</sub>)<sub>5</sub>.**

A. Using the procedure described in Example 22A, but using 6-bromohexanesulfonyl chloride instead of 3-bromopropanesulfonyl chloride, 1-bromo-6-[4-[N-methyl 2-[2-[4-methylsulfonylamino]phenoxy]ethylamino]phenylaminosulfonyl]hexane, 19-2, in which Link is (CH<sub>2</sub>)<sub>5</sub>, is prepared.

B. The above compound (2 mmol) and N-ethyl 2-[4-[2-butylbenzofuran-3-ylcarbonyl]-2,6-diiodophenoxy]ethylamine (19-3), prepared according to procedures described in *Eur. J. Med. Chem.*, 1974, 19-25, are dissolved in MeCN (25 mL). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute NaHCO<sub>3</sub> and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 19-4, in which Link is (CH<sub>2</sub>)<sub>5</sub>.

C. In a similar manner, by employing different bromo compounds 19-2, the corresponding heterovalomers 19-4 are obtained.

## Example 24. (Figure 19)

**Preparation of the dimeric heterovalomer 19-8, in which Link is (CH<sub>2</sub>)<sub>5</sub>.**

A. N-[4-[[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl methanesulfonamide, (E-4031, Table 4) prepared as described in *J. Med. Chem.*, 1990, 903, (10 mmol) is dissolved in 48% HBr in AcOH (50 mL). The solution is heated to 60°C and the progress of the reaction is monitored by tlc. When it is complete, the mixture is cooled and the solvent is removed under reduced pressure. The residue is taken up in water and the solution is basified with aqueous NaOH. The aqueous solution is extracted with CH<sub>2</sub>Cl<sub>2</sub>, and the extract is dried and evaporated. The residue is chromatographed to afford 6-[2-[4-[4-aminobenzoyl-1-piperidyl]ethyl]-2-methylpyridine, 19-5.

B. The above compound 19-5 (2 mmol) is dissolved in  $\text{CH}_2\text{Cl}_2$  (35 mL) and to the solution are added diisopropylethylamine (5 mmol) and 6-bromohexanesulfonyl chloride (2 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute  $\text{NaHCO}_3$  and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford 19-6, in which Link is  $(\text{CH}_2)_5$ .

C. To a solution of the above compound (1 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) is added N-[2-(4-methylsulfonylaminophenoxy)ethyl] 2-(4-methylsulfonylaminophenyl)-ethylamine, 19-7, the preparation of which is described in Example 6A and 6B, (1 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute  $\text{NaHCO}_3$  and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the dimeric product 19-8, in which Link is  $(\text{CH}_2)_5$ .

D. In a similar manner, by employing different bromoalkyl sulfonyl chlorides, as described herein, the corresponding products of Formula 19-8 are obtained.

#### Example 25. (Figure 19)

Preparation of the dimeric heterovalomer 19-11, in which Link is  $(\text{CH}_2)_3$ .

A. Using the procedure of Example 24A, except that 4-bromobutanesulfonyl chloride is employed instead of 6-bromohexanesulfonyl chloride, there is prepared the compound 19-9, in which Link is  $(\text{CH}_2)_3$ .

B. N-Methyl N-(4-aminophenylethyl) 2-[4-(methylsulfonylamino)phenoxy]ethylamine, (8A-1, the preparation of which is described in Examples 4A and 4B) (2 mmol) is dissolved in MeCN (25 mL) and to the solution is added 3-bromopropanesulfonyl chloride (2 mmol). After 6 hours, 10% methanolic methylamine (1 mL) is added. The progress of the reaction is followed by tlc. When it is complete, the

mixture is added to water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to afford N-methyl N-[4-(3-methylaminopropylsulfonyl)-aminophenylethyl]-2-[4-(methylsulfonylamino)phenoxy]ethylamine, 19-10.

5           C.     The product from A above (1 mmol) and the product from B above (1 mmol) are dissolved in EtOH (20 mL). To the mixture is added diisopropylethylamine (20 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with  $\text{CH}_2\text{Cl}_2$ . The extract is dried and evaporated, and the residue is chromatographed to afford the title compound 19-11, in which Link is  $(\text{CH}_2)_3$ .

10           D.     In a similar manner, by employing different bromoalkyl sulfonyl chlorides, as described herein, in step A above, there are obtained the corresponding products of Formula 19-11.

15                                   Example 26. (Figure 20)

**Preparation of 3-cyclopropylmethyl 7-[2-[4-methylsulfonylamino]phenoxy]ethyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-3, in which X is O.**

20           A.     3-Cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, (20-1, in which R is H, the preparation of which is described in Example 14A), (2 mmol) is dissolved in MeCN (20 mL). To the solution is added 2-[4-methylsulfonylamino]phenoxy]ethyl bromide (20-2, in which R is O) (2 mmol) and  $\text{K}_2\text{CO}_3$  (0.5g). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to dilute  $\text{NaHCO}_3$  and extracted with EtOAc. The extract is dried and evaporated and  
25     the residue is chromatographed to afford the title compound 20-3.

          B.     In a similar manner, by employing 2-[4-methylsulfonylamino]phenyl]ethyl bromide 20-2, in which R is a direct bond, in A above, there is obtained the corresponding

product 3-cyclopropylmethyl 7-[2-[4-methylsulfonylaminophenyl]ethyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-3, in which X is a direct bond.

Example 27. (Figure 20)

5 Preparation of 3,8-di-[2-[4-methylsulfonylaminophenoxy]ethyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-5, in which X is O.

A. 3,8-Dibenzyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-4, in which R<sub>1</sub> and R<sub>2</sub> are benzyl, the preparation of which is described in European Patent 461574, (5 mmol) is dissolved in EtOH (25 mL). 10% Pd/C (50 mg) and ammonium formate (200 mg) are added. The progress of the reaction is followed by tlc. When it is complete, the solution is filtered and the solvent is removed under vacuum to afford 9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-4, in which R<sub>1</sub> and R<sub>2</sub> are H.

15 B. The above compound (1 mmol) is dissolved in MeCN, and to the solution is added diisopropylethylamine (5 mmol) and 2-[4-methylsulfonylaminophenoxy]ethyl bromide (0.5 mmol). The progress of the reaction is monitored by tlc. When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 20-5, in which X is O.

20

C. In a similar manner, by employing 2-[4-methylsulfonylaminophenyl]ethyl bromide in B above, there is obtained the corresponding product 3,8-di-[2-[4-methylsulfonylaminophenyl]ethyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane 20-5, in which X is a direct bond.

25

## Example 28. (Figure 20)

Preparation of 3-cyclopropylmethyl-7-[4-[2-[4-methylsulfonylamino]phenoxy]-ethylmethylamino]butyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-5, in which X is O.

5  
A. 3-Cyclopropylmethyl-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, (20-1, in which R is H), (5 mmol) and 1,4-dibromobutane (5 mmol) are dissolved in EtOH (30 mL). The progress of the reaction is followed by tlc. When it is complete, N-methyl 2-(4-methylsulfonylamino]phenoxy)ethylamine (20-6, in which X is O), (5 mmol) is added. The  
10 progress of the reaction is followed by tlc. When it is complete, the mixture is added to dilute NaHCO<sub>3</sub> and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound 20-5, in which X is O.

15 B. In a similar manner, by employing 2-[4-methylsulfonylamino]phenyl]ethyl bromide in A above, there is obtained the corresponding product 3-cyclopropylmethyl-7-[4-[2-[4-methylsulfonylamino]phenyl]-ethylmethylamino]butyl]-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane, 20-5, in which X is a direct bond.

20 C. In a similar manner, by employing different dibromo compounds in place of 1,4-dibromobutane, there are obtained the corresponding products similar to 20-7.

## Example 29. (Figure 20)

Preparation of 1,3,5-tri-[2-[4-(methylsulfonylamino)phenoxy]ethylmethylamino-methyl]benzene, 20-9, in which X is O.

25 A. N-Methyl 2-[(4-methylsulfonylamino)phenoxy]ethylamine (20-6, in which X is O), (3 mmol) and 1,3,5-tri-(bromomethyl) benzene (20-8), (1 mmol) are dissolved in MeCN (25 mL) containing K<sub>2</sub>CO<sub>3</sub> (0.5g). The progress of the reaction is monitored by tlc.



When it is complete, the mixture is added to water and extracted with EtOAc. The extract is dried and evaporated and the residue is chromatographed to afford the title compound, 20-9, in which X is O.

5           B.     In a similar manner, by employing N-methyl 2-[(4-methylsulfonylamino)phenyl]-ethylamine, (20-6, in which X is a direct bond) there is obtained the corresponding product 1,3,5-tri-[2-[4-(methylsulfonylamino)phenyl]ethylmethylamino-methyl]benzene, 20-9, in which X is a direct bond.

10

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective  
15           spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

20           All of the publications, patent applications and patents cited in this application are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

Table 1: Multiple Activities of Antiarrhythmic Agents

Drug	Channels					Receptors		
	Na			Ca	K	$\alpha$	$\beta$	$M_2$
	Fast	Med.	Slow					
<b>Class I</b>								
Lidocaine	•							
Mexiletine	•							
Tocainide	•							
Moricizine	♦							
Procainamide		♦			■			
Disopyramide		♦			■			•
Quinidine		♦			■	•		•
Propafenone		♦					■	
Flecainide			♦		•			
Encainide			♦					
<b>Class IV</b>								
Bepiridil	•			♦	■			
Verapamil	•			♦		■		
Diltiazem				■				
<b>Class III</b>								
Bretylum					♦	★	★	
Sotalol					♦		♦	
Amiodarone	•			•	♦	■	■	
Afinidine					■			

Potency of block: Low ■ Moderate ♦ High ★ Biphasic Action

Table 2: Activity of Potassium Channel Ligands

Drug	Development Stage	I <sub>kr</sub>	I <sub>Ks</sub>	I <sub>to</sub>	Na <sub>slow</sub>	Use Dependence
Amiodarone	Approved	•	•			None
Dofetilide	Phase 3	•				Reverse
Sematilide	Phase 3	•				Reverse
E-4031	Phase 2	•				Reverse
Azimilide	Phase 3	•	•			Reverse
Ambasilide	Terminated	•	•			None
Ibutilide	Approved	•			1	None
Tedisamil	Phase 3	•		•		Reverse

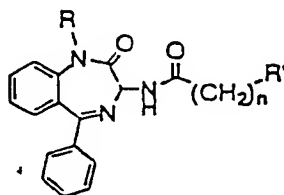
Table 3: Properties of Potassium-Channel Blockers

Drug	Trade Name	Developer	Status	IC50 ( $\mu$ M)			Bioavail.	Elim $t_{1/2}$
				IK <sub>r</sub>	IK <sub>r</sub>	I <sub>to</sub>		
Dofetilide	Tikosyn	Pfizer	Phase 3	0.01			100%	7-13 hrs.
E-4031		Eisai	Phase 2	0.4			90%	
Sematilide		Schering AG	Phase 3	25			50%	
Ambasilide		BASF	Term.					
Azimilide	Stedcor	P&G	Phase 3	0.2	2			
Tedisamil		Solvay	Phase 3	<10		5		
Dronedarone		Sanofi	Phase 2					
Ibutilide	Corvert	Pharmacia	Apprvd	0.016			<5%	4-8 hrs.
d-Solatol		Bristol-Myers	Pre-reg	100			100%	
Amiodarone	Cordarone	Wyeth-Ayerst	Apprvd				22-86%	3-21 hrs.

Table 4: Potassium Channel Ligands

UK66,914 (I <sub>K</sub> )			
RP58866 (I <sub>K1</sub> )		Amiodarone	
Tacrine (I <sub>K</sub> and I <sub>K1</sub> )			
4-Aminopyridine (I <sub>to</sub> )			
Glibenclamide (I <sub>K(ATP)</sub> )			
RP49358 (I <sub>K(ATP)</sub> opener)			
$\text{CH}_3\text{C}(=\text{O})\text{NH}-\text{C}_6\text{H}_4-\text{C}(=\text{O})\text{NH}-\text{CH}_2-\text{CH}_2-\text{N}(\text{C}_2\text{H}_5)_2$			
<u>N-Acetyl Procainamide (NAPA)</u>			
$\text{Cl}-\text{C}_6\text{H}_4-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{N}(\text{C}_2\text{H}_5)_2$			
<u>Clofilium</u>			

Table 4: Potassium Channel Ligands (continued)

Effect of Substitution on  $I_K$  PotencyL - 768,673  
and analogs

compd <sup>a</sup>	abs conf	R	n	R'	$I_K$ IC <sub>50</sub> (nM) <sup>b</sup>	$I_K$ IC <sub>50</sub> (nM) <sup>c</sup> or % inh at concn (nM)	CCK-B IC <sub>50</sub> (nM) <sup>d</sup>	mp °C
22 <sup>e</sup>	R	Me	0	<i>m</i> -NHPbCH <sub>3</sub>	214	5000	2 ± 0.3	
2b <sup>20</sup>	S	Me	0	<i>m</i> -NHPbCH <sub>3</sub>	10000	10000	151 ± 12	
3	R	Me	0	phenyl	600 <sup>d</sup>	23% at 1000	>1000	224-225
4	R	Me	0	3,5-dichlorophenyl	45 <sup>d</sup>	17% at 1000		179-180
5	R	Me	1	phenyl	300 <sup>d</sup>	31% at 1000	>1000	241-242
6	R	Me	1	2,4-dichlorophenyl	35			209-210
7	R	Me	1	2,4-bis(trifluoromethyl)phenyl	140			100-103
8	R	Me	2	phenyl	200 <sup>d</sup>	32% at 1000	>1000	179
9	R	Me	2	2,4-dichlorophenyl	14 <sup>d</sup>	31% at 100	>1000	92-95
10	R	Me	0	CH=CH-2,4-dichlorophenyl	6 <sup>d</sup>	1500	>1000	137-139
11	R	Me	0	CH(OH) <sub>2</sub> CH <sub>2</sub> Ph	2800 <sup>d</sup>	8800	>1000	84-86
12	R	Me	2	4-aminophenyl	4400 <sup>d</sup>		>1000	175-178
13	R	Me	2	4-acetamidophenyl	>10000		>1000	138-142
14	R	Me	2	cyclohexyl	10 <sup>d</sup>	1000	>1000	144-145
15	RS	H	2	cyclohexyl	1000			192-193
16	RS	Me <sub>2</sub> NCH <sub>2</sub> CH <sub>3</sub>	2	2,4-dichlorophenyl	520 <sup>d</sup>	100	>1000	199-201
17	R	<i>i</i> -Pr	2	cyclohexyl	20 <sup>d</sup>		>1000	154-155
18	R	<i>i</i> -Pr	0	3,5-dichlorophenyl	6			140-141
19	S	<i>i</i> -Pr	0	3,5-dichlorophenyl	110	6% at 1000		140-141
20	R	<i>i</i> -Pr	1	3,5-dichlorophenyl	10 <sup>d</sup>		>1000	90-96
21	R	F <sub>3</sub> CCH <sub>3</sub>	0	3,5-dichlorophenyl	11 <sup>d</sup>	25% at 100		140-143
22	R	F <sub>3</sub> CCH <sub>3</sub>	1	3,5-dichlorophenyl	30	4000		93-100
23	R	F <sub>3</sub> CCH <sub>3</sub>	1	2,4-dichlorophenyl	9	2400		143-145
24	S	F <sub>3</sub> CCH <sub>3</sub>	1	2,4-bis(trifluoromethyl)phenyl	60 <sup>d</sup>	35% at 1000	>1000	
1	R	F <sub>3</sub> CCH <sub>3</sub>	1	2,4-bis(trifluoromethyl)phenyl	6 <sup>d</sup>	6000	>1000	132-134

Table 5: Principal Cardiac K<sup>+</sup> Currents and Some Drugs that Block Them

Current	Drugs that Block Current	Reference
I <sub>K</sub>	UK66,914, dofetilide, sematilide, <i>d</i> -solatol	Argentieri, 1992; Carmeliet, 1985; Gwilt, et al., 1990, 1991
I <sub>K1</sub>	RP58866, RP62719	Escande, et al., 1992; Imoto, et al., 1987
I <sub>TO1</sub>	Tedisamil	Dukes, et al., 1990
I <sub>K(ATP)</sub>	Glibenclamide, 5-hydroxydecanoate	Kantor, et al., 1990; Notsu, et al., 1989; 1992

## WHAT IS CLAIMED IS:

1. A multibinding compound comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel.

2. The multibinding compound of Claim 1 wherein said ligand is selected from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide, ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

3. The multibinding compound of Claim 1 which has 2 ligands.

4. A multibinding compound represented by Formula I:



where each L is a ligand that may be the same or different at each occurrence;

X is a linker that may be the same or different at each occurrence;

p is an integer of from 2 to 10; and

q is an integer of from 1 to 20;

wherein each of said ligands comprises a ligand domain capable of binding to a K<sup>+</sup> channel.

5. The multibinding compound of Claim 4, wherein q is less than p.

6. The multibinding compound of Claim 4 wherein said ligand is selected from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium,



5 melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide, ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

7. The multibinding compound of Claim 4 wherein  $p$  is 2 and  $q$  is 1.

10 8. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of one or more multibinding compounds, or pharmaceutically acceptable salts thereof, comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a  $K^+$  channel  
15 of a cell mediating mammalian diseases or conditions, thereby modulating the diseases or conditions.

9. The pharmaceutical composition of Claim 8 wherein said ligand is selected from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide,  
20 almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide, ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and  
25 amiodarone.

10. The pharmaceutical composition of Claim 8 which has 2 ligands.

11. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutically effective amount of one or more multibinding compounds represented by Formula I:



5 and pharmaceutically acceptable salts thereof,

where each L is a ligand that may be the same or different at each occurrence;

X is a linker that may be the same or different at each occurrence;

p is an integer of from 2 to 10; and

q is an integer of from 1 to 20;

10 wherein each of said ligands comprises a ligand domain capable of binding to a K<sup>+</sup> channel of a cell mediating mammalian diseases or conditions, thereby modulating the diseases or conditions.

12. The pharmaceutical composition of Claim 11 wherein said ligand is selected  
15 from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide,  
20 ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

13. The pharmaceutical composition of Claim 11 which has 2 ligands.

25 14. A method for modulating the activity of a K<sup>+</sup> channel in a biologic tissue, which method comprises contacting a tissue having a K<sup>+</sup> channel with a multibinding compound, or a pharmaceutically acceptable salt thereof, under conditions sufficient to produce a change in the activity of the channel in said tissue, wherein the multibinding compound comprises 2 to 10 ligands which may be the same or different and which are

covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel.

15. The method of Claim 14 wherein said ligand is selected from the group  
5 consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide,  
10 ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

16. The method of Claim 14 wherein the multibinding compound has 2 ligands.

15 17. A method for treating a disease or condition in a mammal resulting from an activity of a K<sup>+</sup> channel, which method comprises administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds, or pharmaceutically acceptable salts thereof, comprising 2 to 10 ligands which may be the same  
20 or different and which are covalently attached to a linker or linkers, which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a K<sup>+</sup> channel of a cell mediating mammalian diseases or conditions.

18. The method of Claim 17 wherein said ligand is selected from the group  
25 consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afinidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide,

ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

19. The method of Claim 17 wherein the multibinding compound has 2 ligands.

20. A method for treating a disease or condition in a mammal resulting from an activity of a  $K^+$  channel, which method comprises administering to said mammal a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and one or more multibinding compounds represented by Formula I:



and pharmaceutically acceptable salts thereof,

where each L is a ligand that may be the same or different at each occurrence;

X is a linker that may be the same or different at each occurrence;

p is an integer of from 2 to 10; and

q is an integer of from 1 to 20;

wherein each of said ligands comprises a ligand domain capable of binding to a  $K^+$  channel of a cell mediating mammalian diseases or conditions.

21. The method of Claim 20 wherein said ligand is selected from the group consisting of quinidine, glibenclamide, procaine, tetraethyl ammonium, clofilium, melperone, pinacidil, WAY-123,398, cromakalim, propofol, thiopentone, risotilide, almokalant, bretylium, N-acetylprocainamide, tacrine, UK66,914, RP58866, 4-aminopyridine, RP49356, afimidine, chromanol 293B, L-768,673 and its analogs, bethanidine, disopyramide, desethylamiodarone, NE-10064, artilide, dofetilide, E-4031, sematilide, ambasilide, azimilide, tedisamil, dronedarone, ibutilide, sotalol, benzodiazepine analogs and amiodarone.

22. The method of Claim 20 wherein the multibinding compound has 2 ligands.

23. A method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

5 (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

10 (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

15 24. A method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

20 (b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

25 (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

25. The method according to Claim 23 or 24 wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

5

26. The method according to Claim 25 wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

27. The method according to Claim 26 wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

10

28. The method according to Claim 27 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

15

29. The method according to Claim 23 or 24 wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

30. The method according to Claim 29 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

20

31. The method according to Claim 23 or 24 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and amphiphilic linkers.

25

32. The method according to Claim 31 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

33. The method according to Claim 32 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

34. The method according to Claim 23 or 24 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

35. The method according to Claim 34 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

36. The method according to Claim 23 or Claim 24 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

37. The method according to Claim 23 or Claim 24 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

38. A library of multimeric ligand compounds which may possess multivalent properties for potassium channels, which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the

library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

39. A library of multimeric ligand compounds which may possess multivalent properties for potassium channels, which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

40. The library according to Claim 38 or Claim 39 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and amphiphilic linkers.

41. The library according to Claim 40 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

42. The library according to Claim 41 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

43. The library according to Claim 38 or 39 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.



44. The library according to Claim 43 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive  
5 functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

45. The library according to Claim 38 or Claim 39 wherein the multimeric  
10 ligand compound library comprises homomeric ligand compounds.

46. The library according to Claim 38 or Claim 39 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

15 47. An iterative method for identifying multimeric ligand compounds possessing multibinding properties for potassium channels, which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or  
20 mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

25 (b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

5 (e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted or are consistent with imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

10 (g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

48. The method according to Claim 47 wherein steps (e) and (f) are repeated from 2-50 times.

15 49. The method according to Claim 47 wherein steps (e) and (f) are repeated from 5-50 times.

1/23

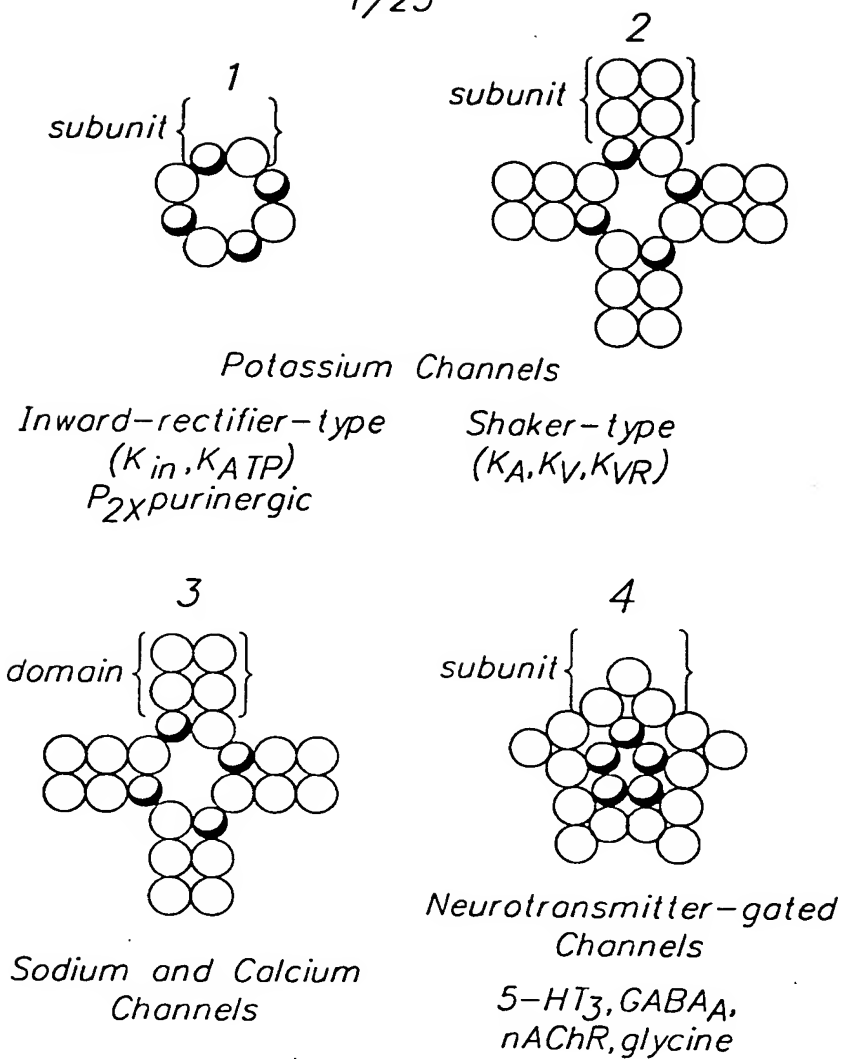
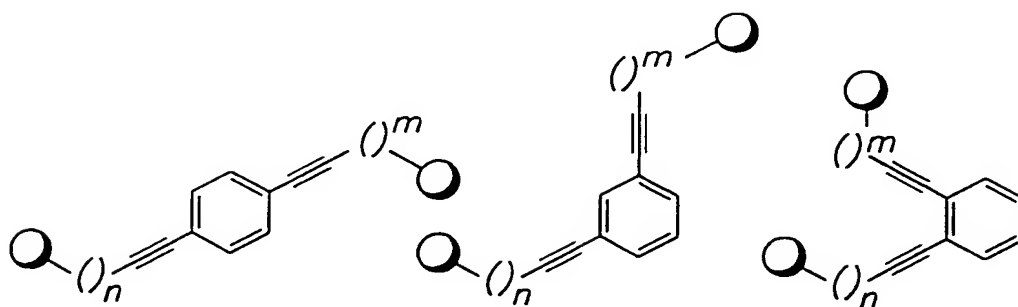


FIG. 1



= ligand

 $n+m+\text{core} < 100$  atoms

FIG. 2A

2/23

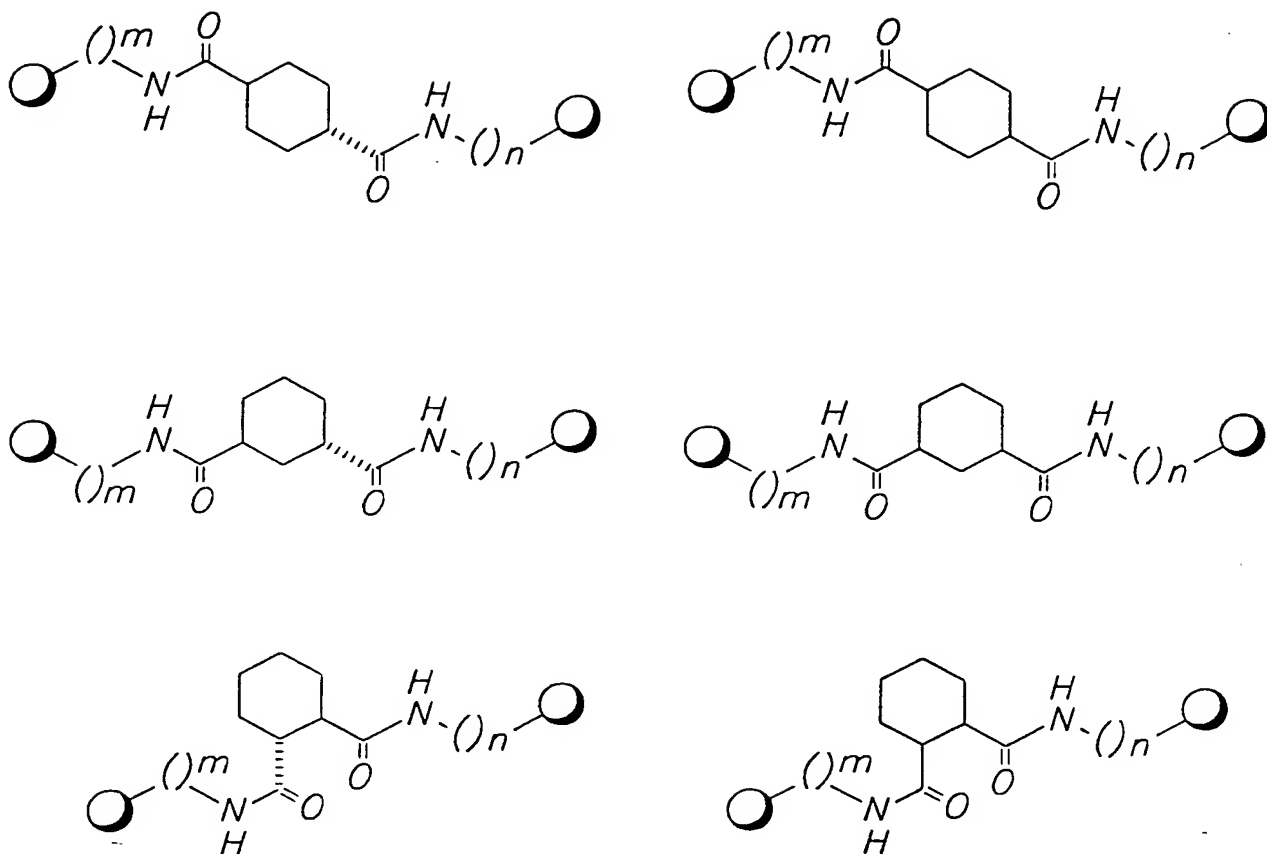


FIG. 2B

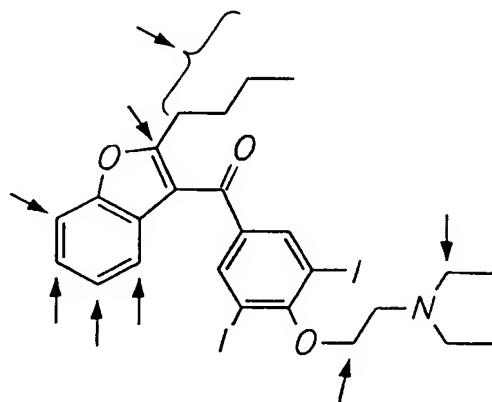


FIG. 5

**FIG. 3A**

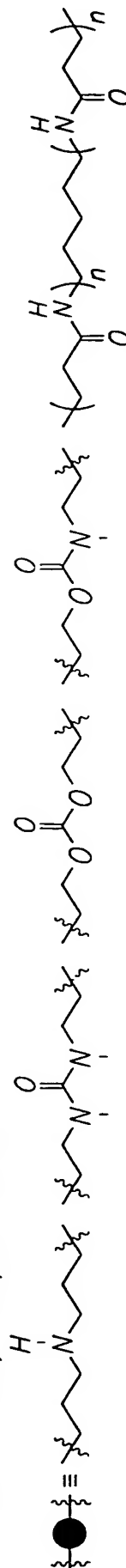
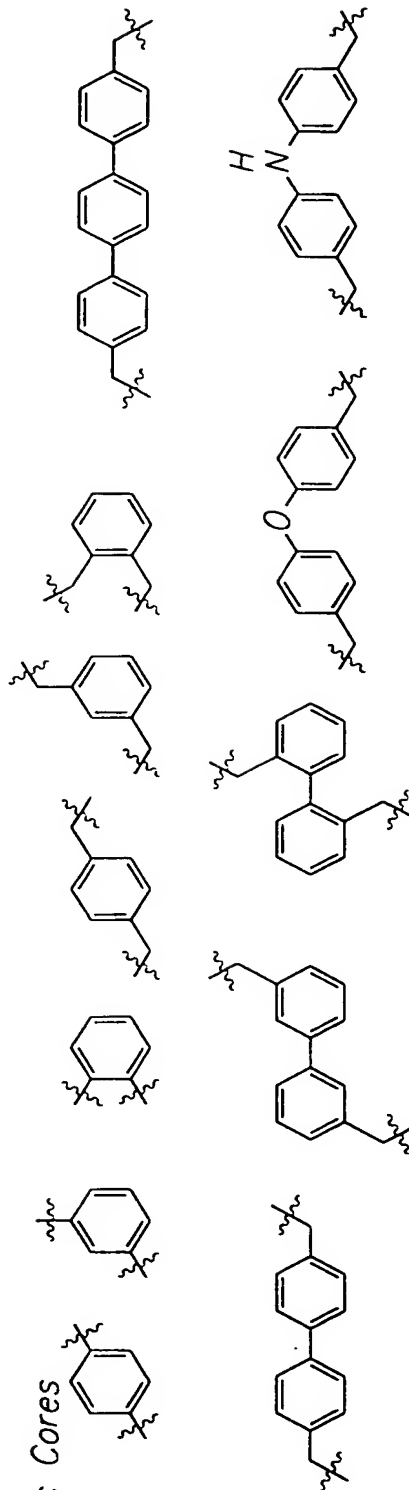
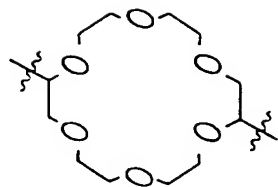
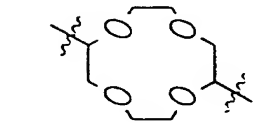
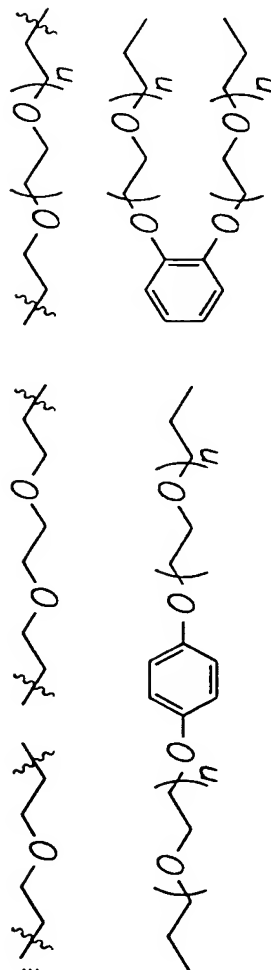
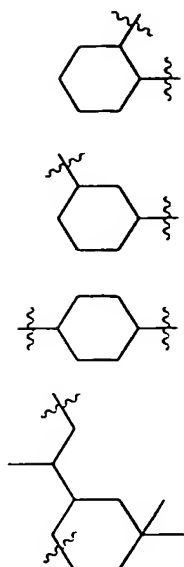
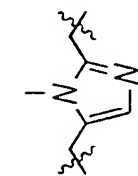
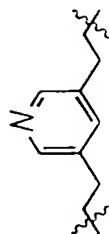
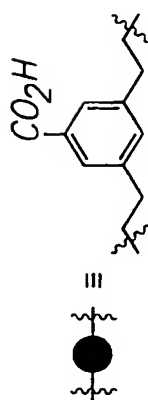
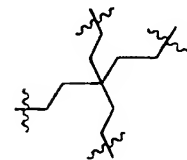
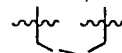
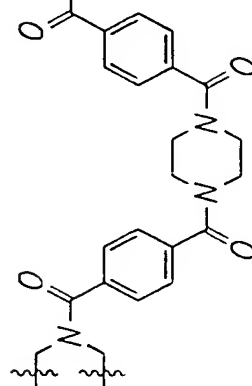
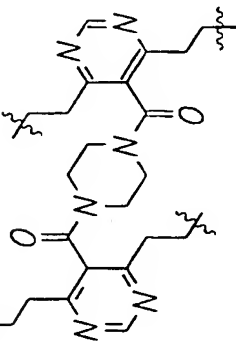
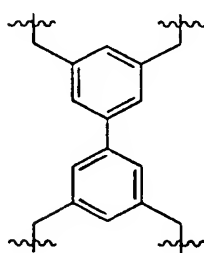
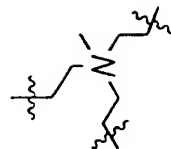
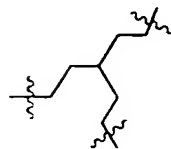
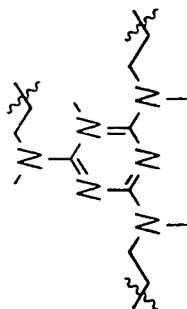
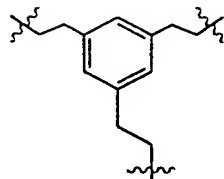
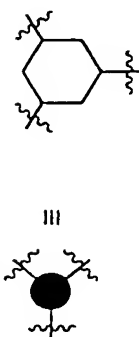


FIG. 3B

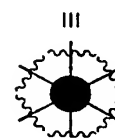
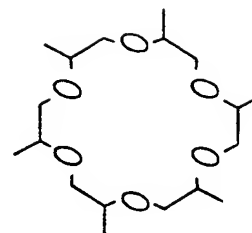
Acidic/Basic Cores



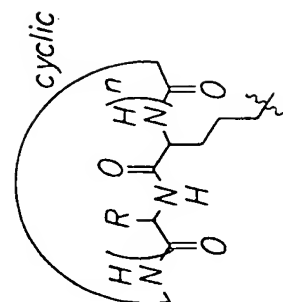
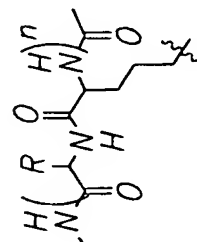
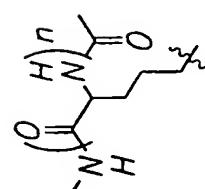
Higher Order Cores



4/23

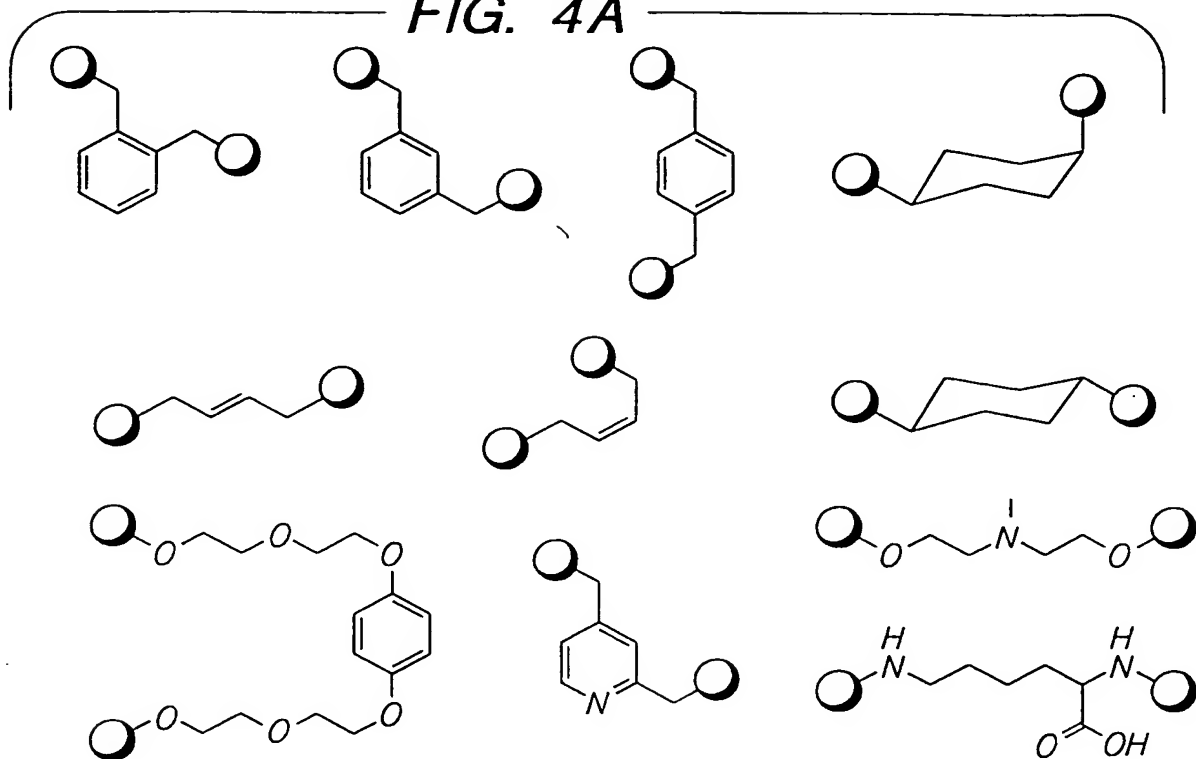


Peptidic Cores

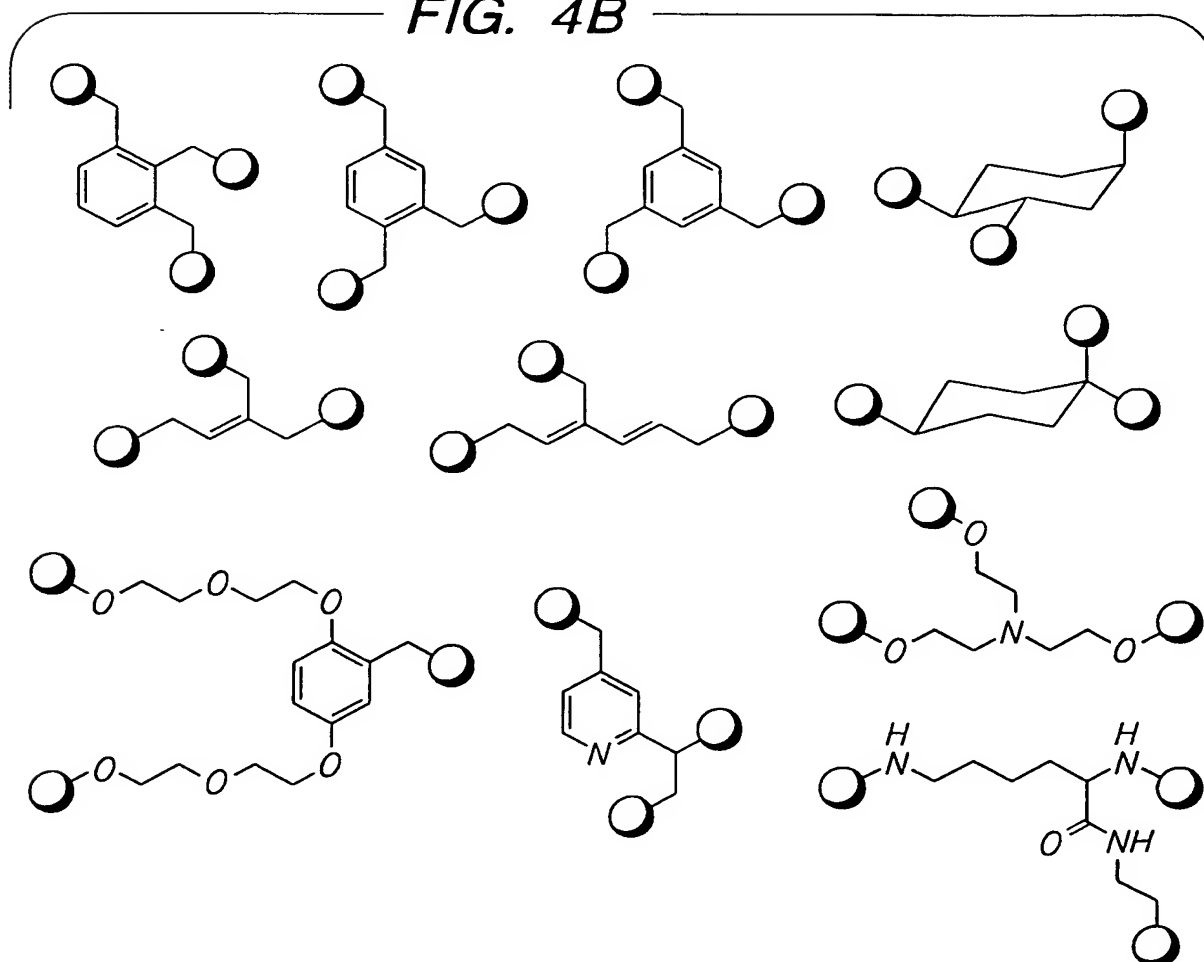


5/23

**FIG. 4A**



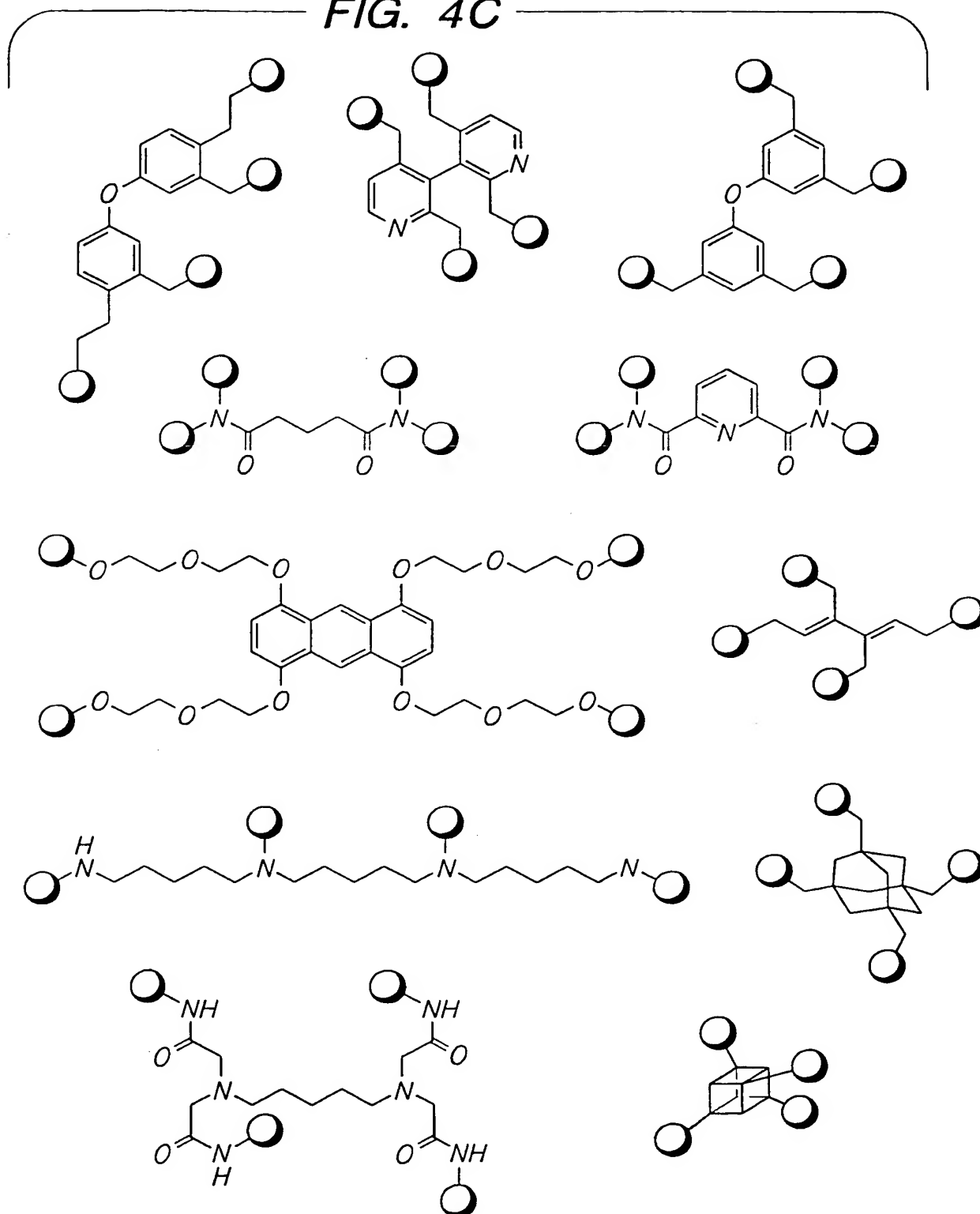
**FIG. 4B**



SUBSTITUTE SHEET (RULE 26)

6/23

FIG. 4C

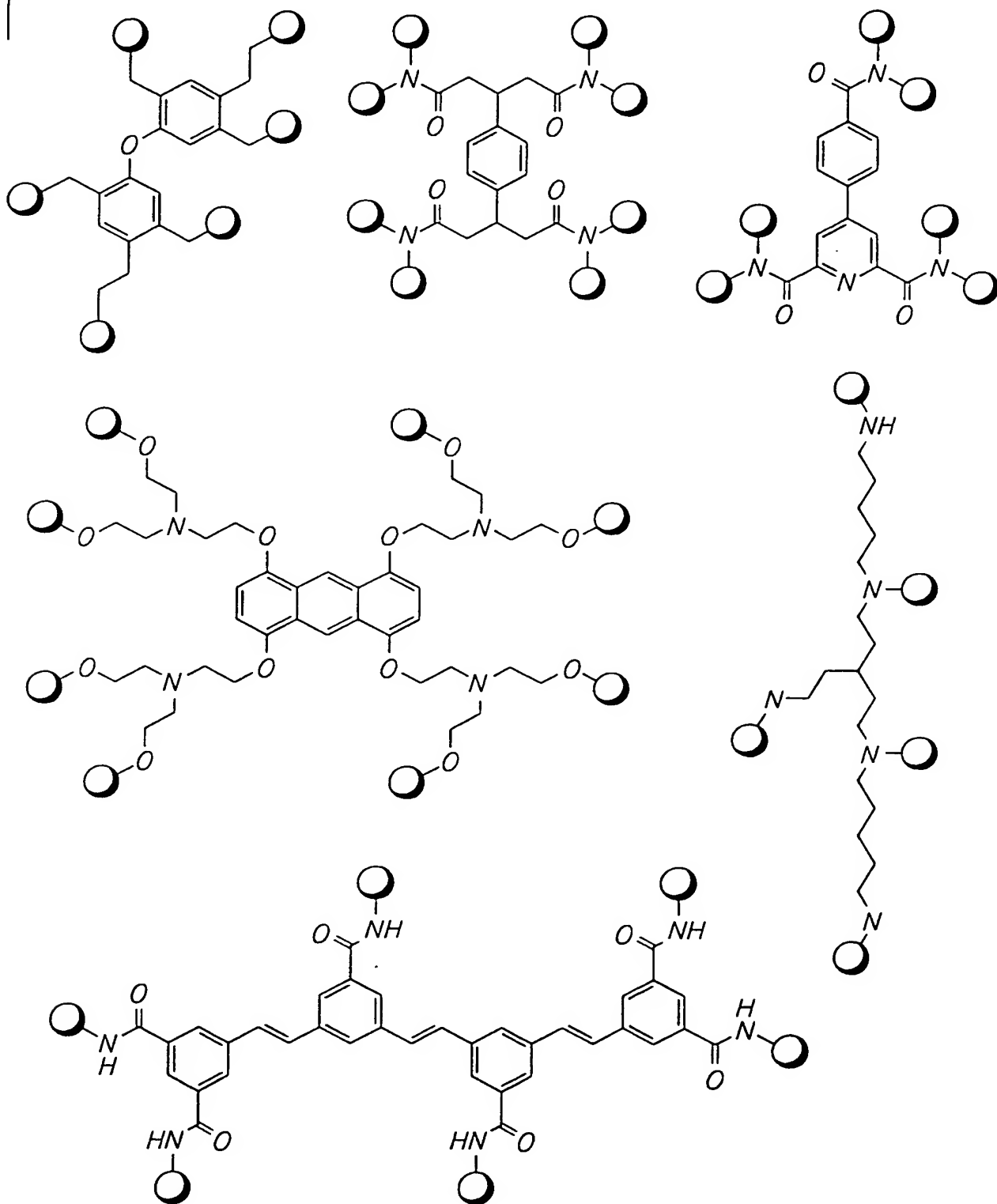


SUBSTITUTE SHEET (RULE 26)



7/23

FIG. 4D



8/23

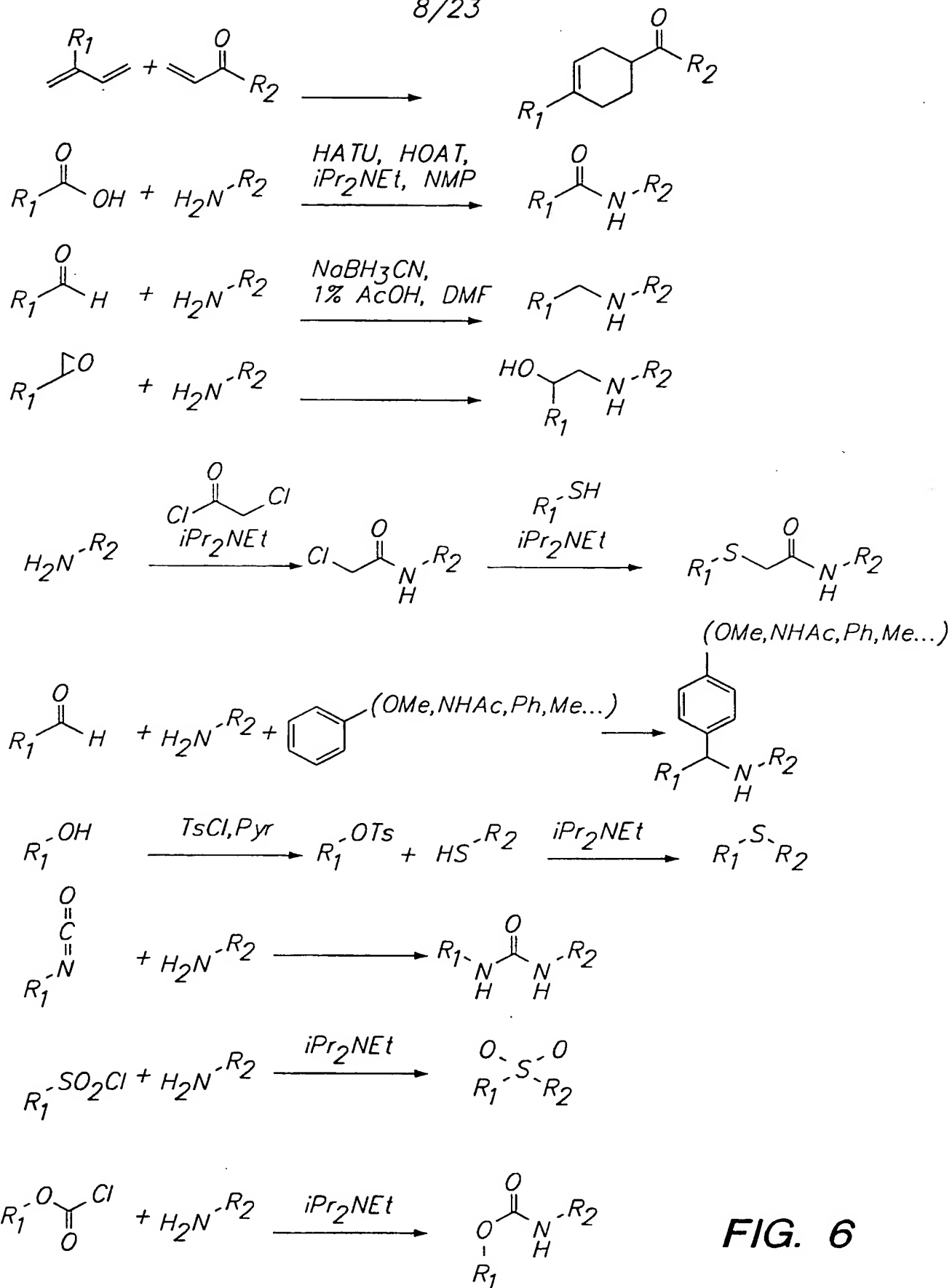


FIG. 6

9/23

## Reaction Schemes for Amiodarone and Dronedarone

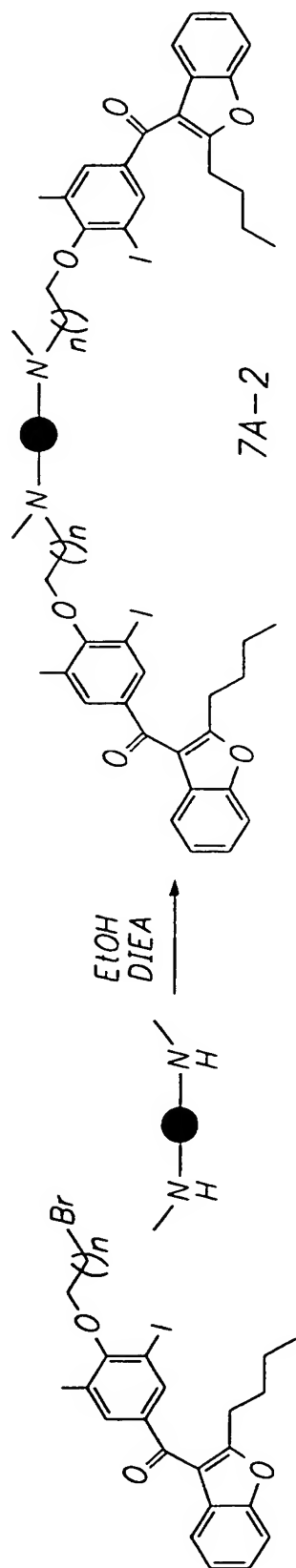


FIG. 7A

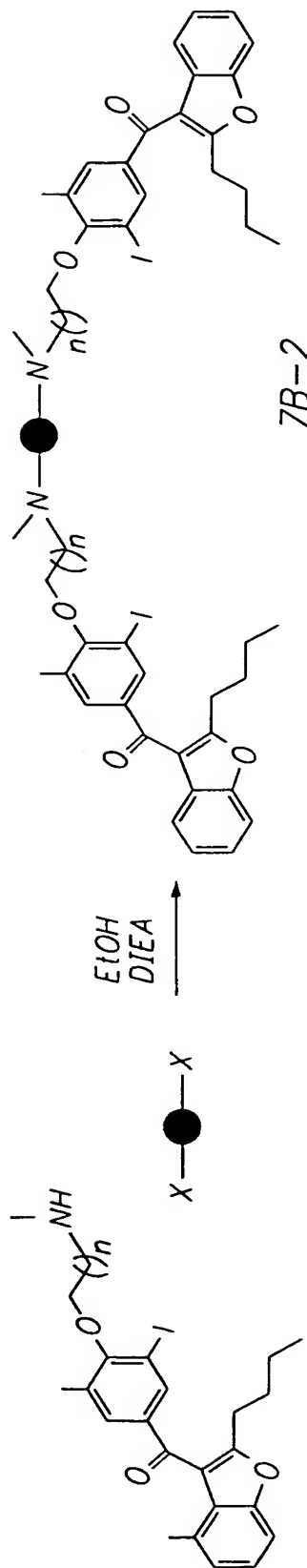


FIG. 7B

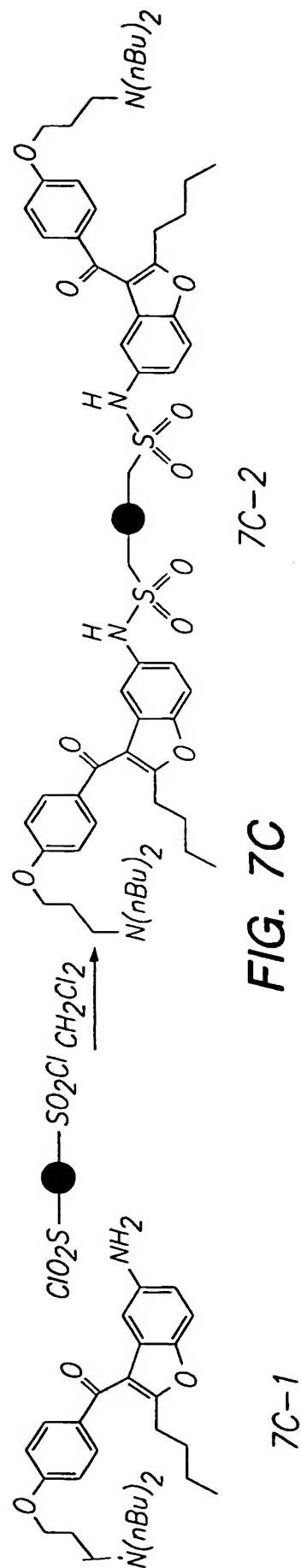


FIG. 7C

10/23

## Reaction Schemes for Bivalent Dofetilide Compounds

FIG. 8A

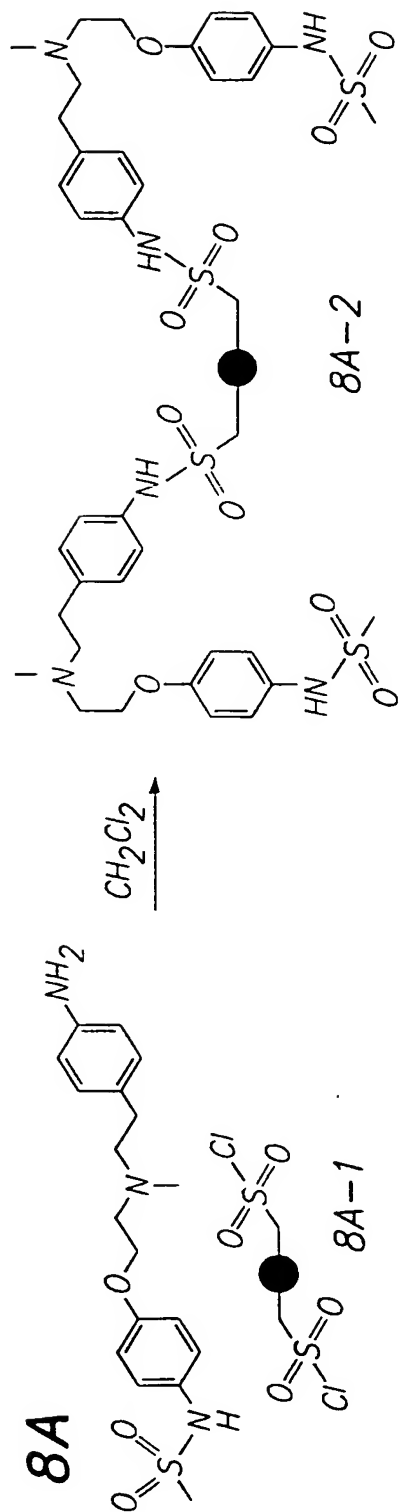


FIG. 8B

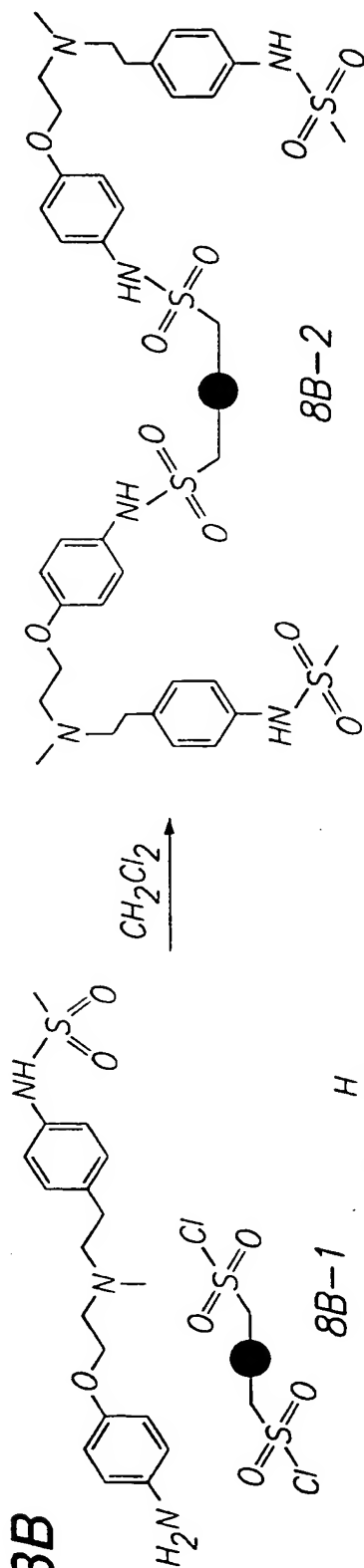
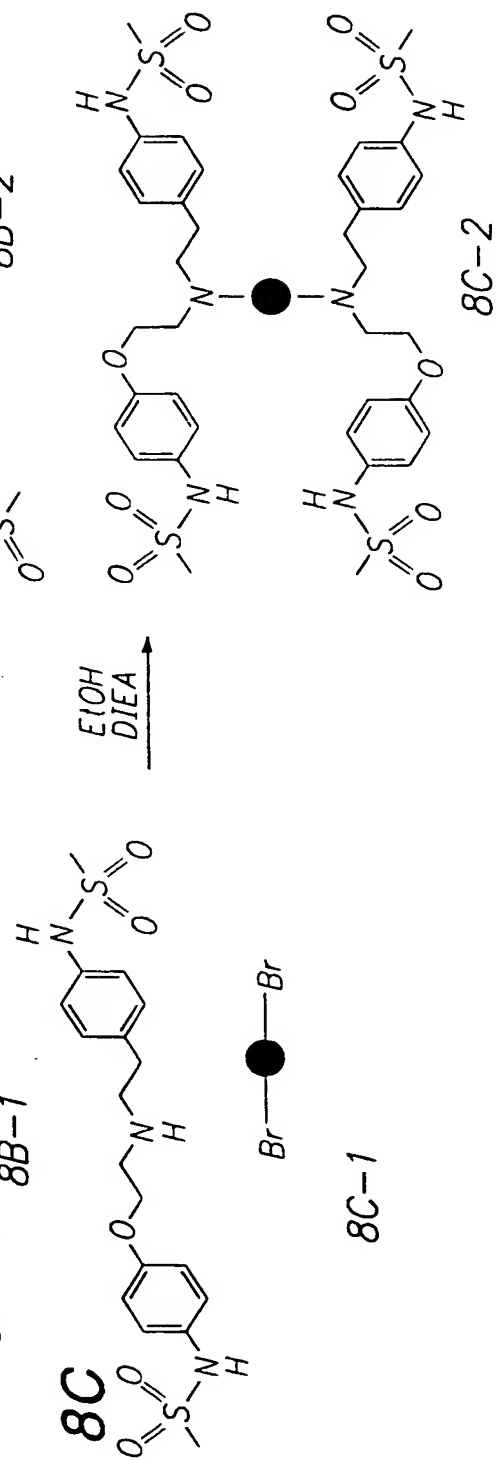
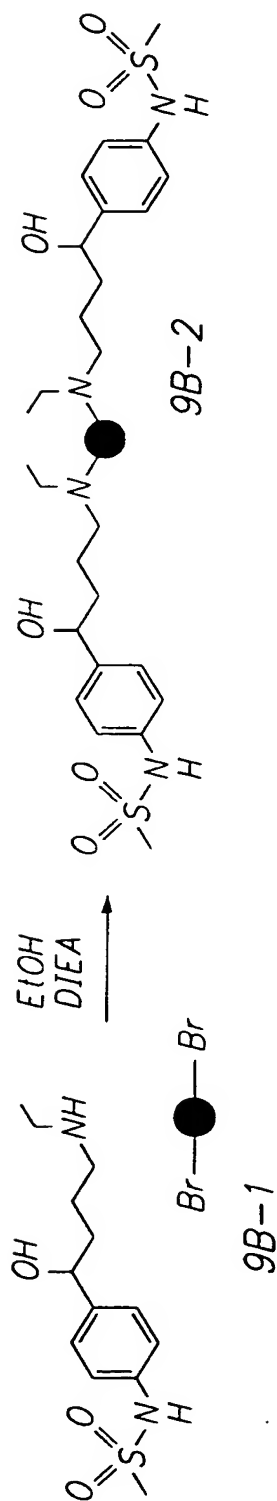
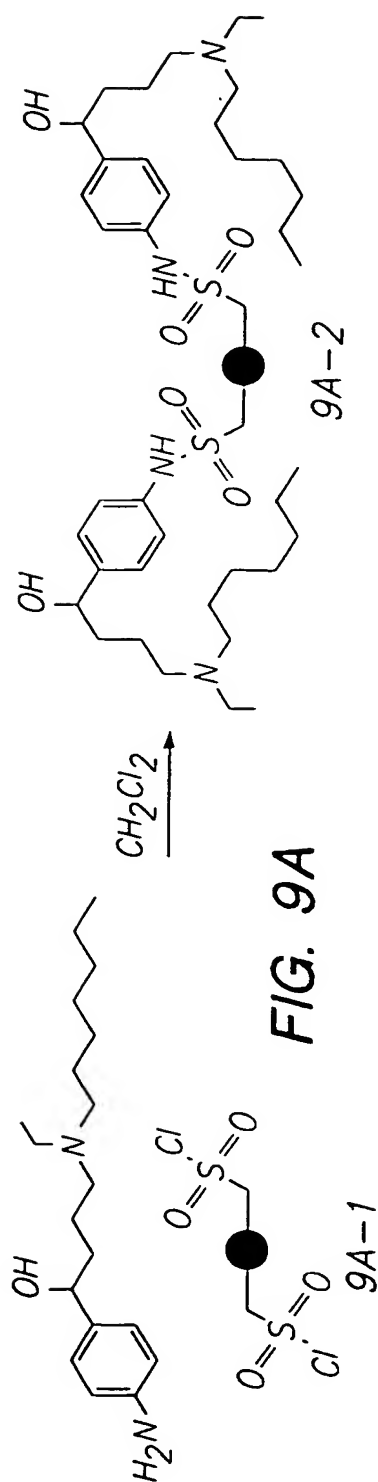


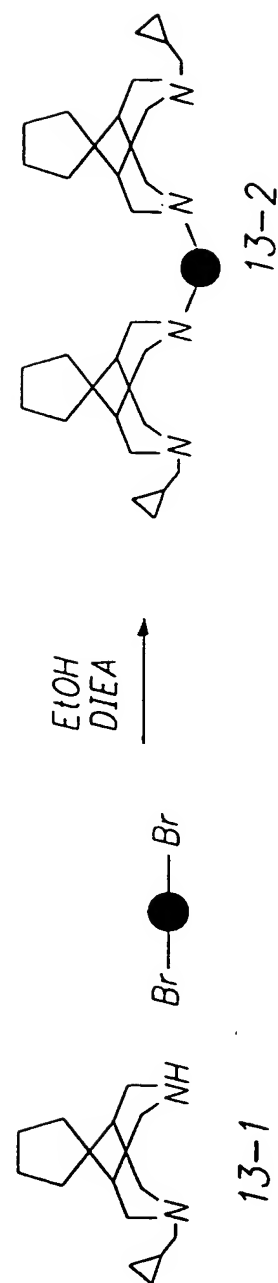
FIG. 8C



11/23

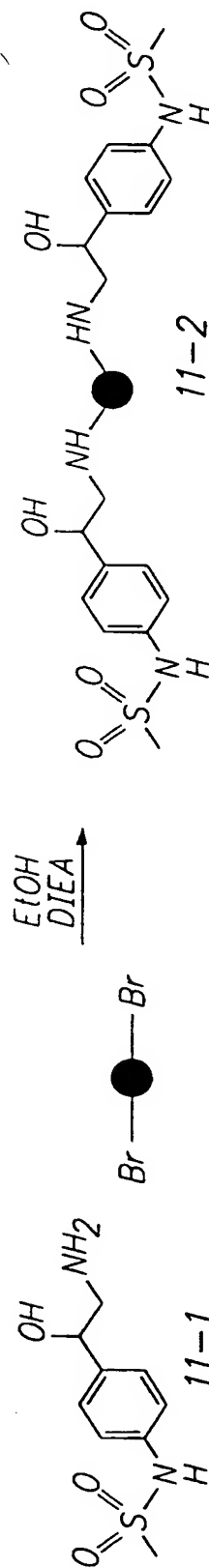
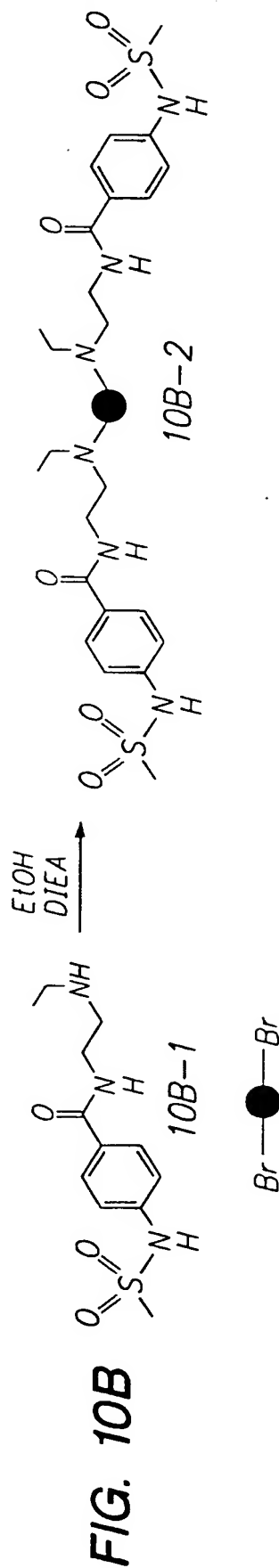
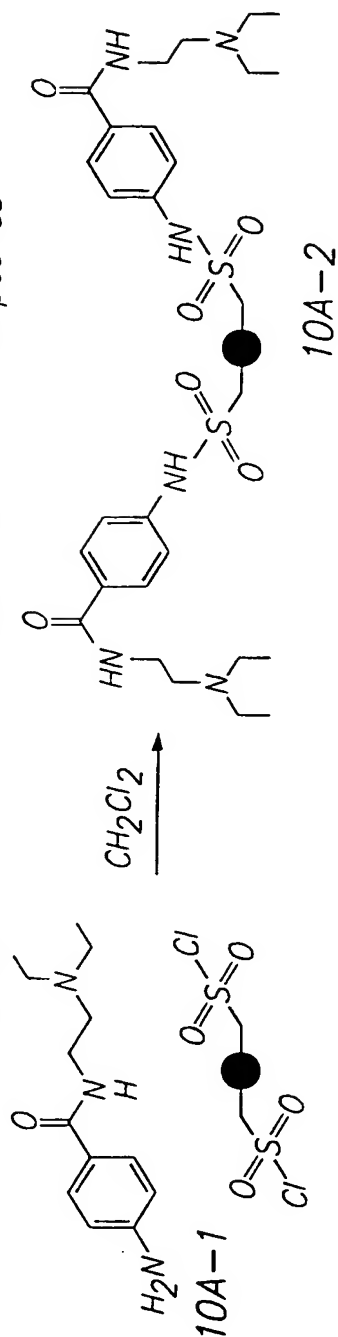


**FIG. 9B** Reaction Schemes for Bivalent Ibutilide Compounds



**FIG. 13** Reaction Schemes for Bivalent Tedasimil Compounds

## Reaction Schemes for Bivalent Sematilde Compounds

**FIG. 11** Reaction Schemes for Bivalent Sotalol Compounds

13/23

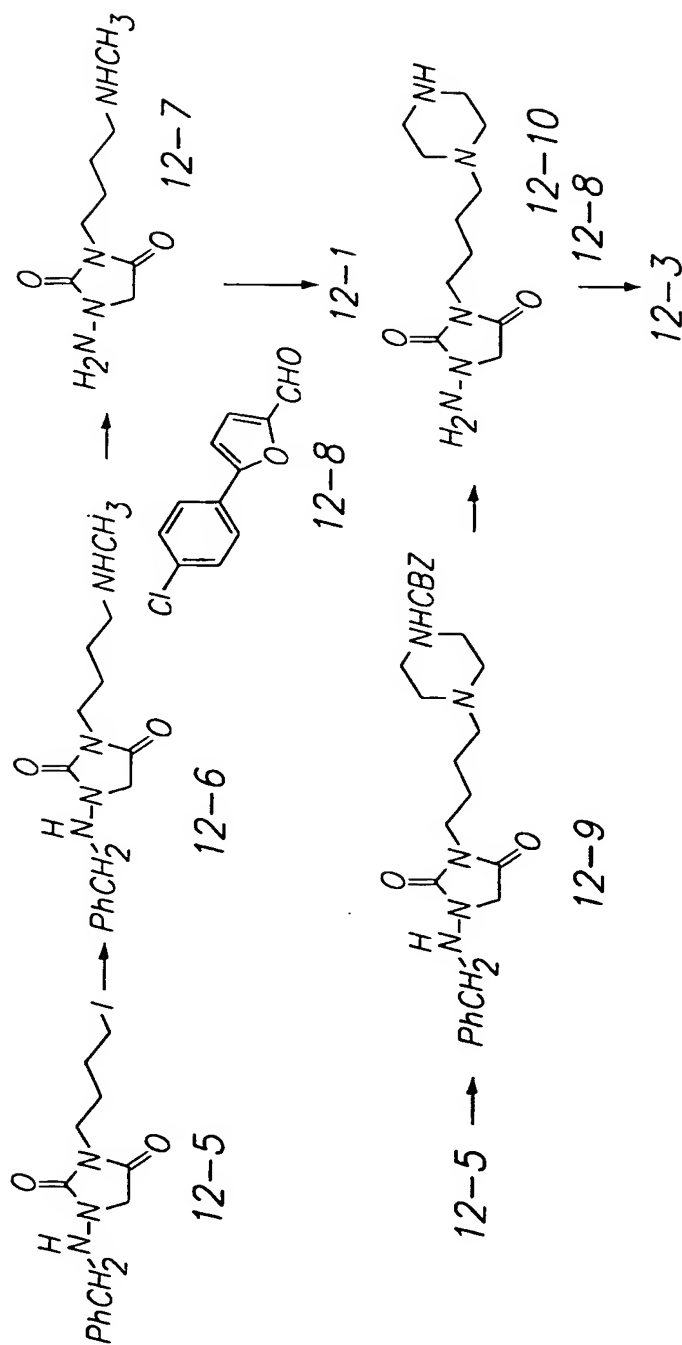
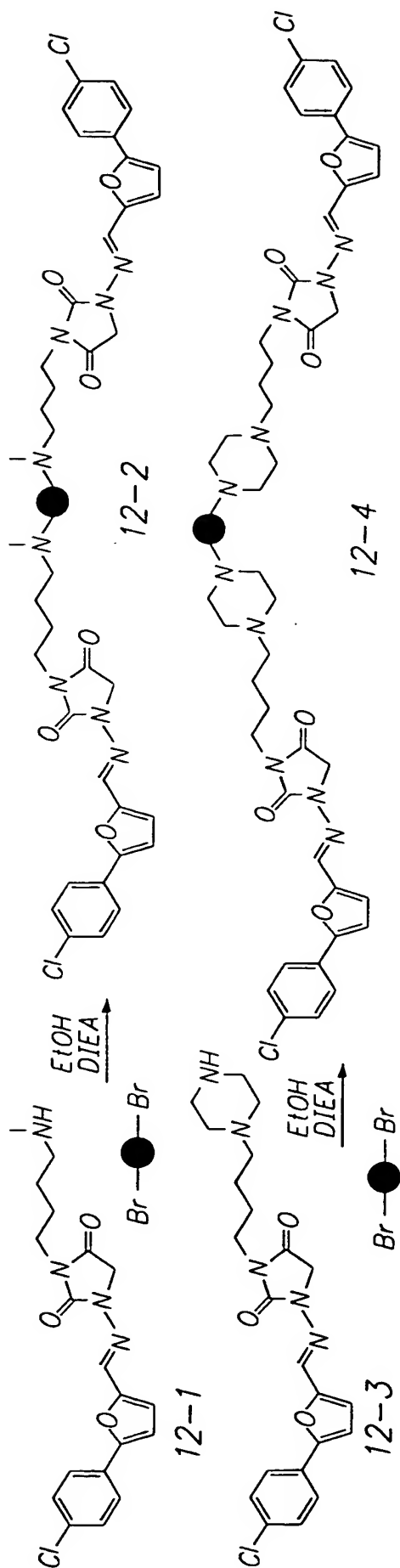


FIG. 12 Reaction Schemes for Bivalent Azimilide Compounds

Example 15

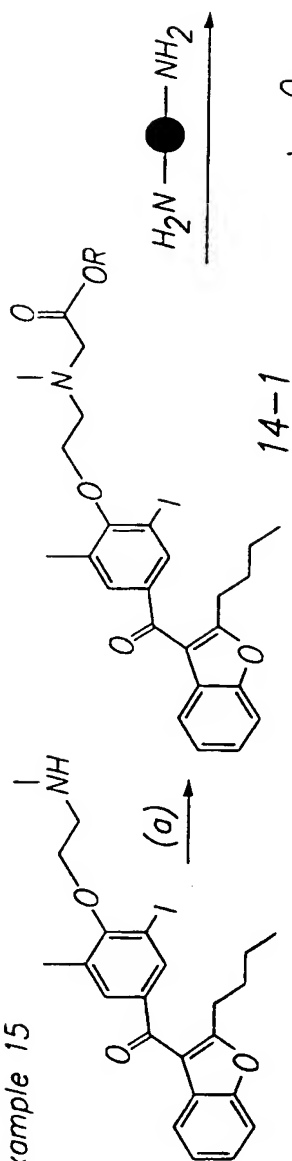


FIG. 14A

14-1

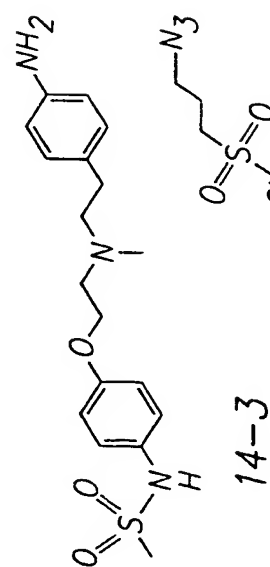


7B-1

(a)  $\text{BrCH}_2\text{COOEt}$ , DIEA,  $\text{CH}_2\text{Cl}_2$  (b)  $\text{LiOH}$ ,  $\text{THF}$ ,  $\text{H}_2\text{O}$ 

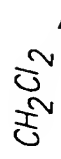
14-2

Example 16



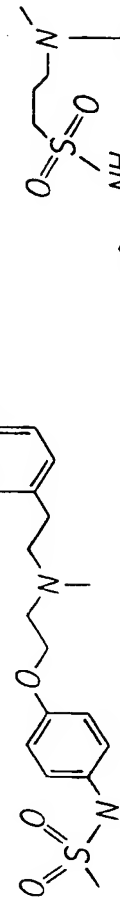
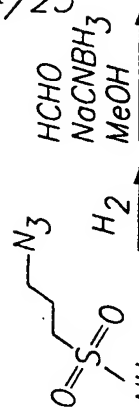
14-3

14-4



14-5

14/23

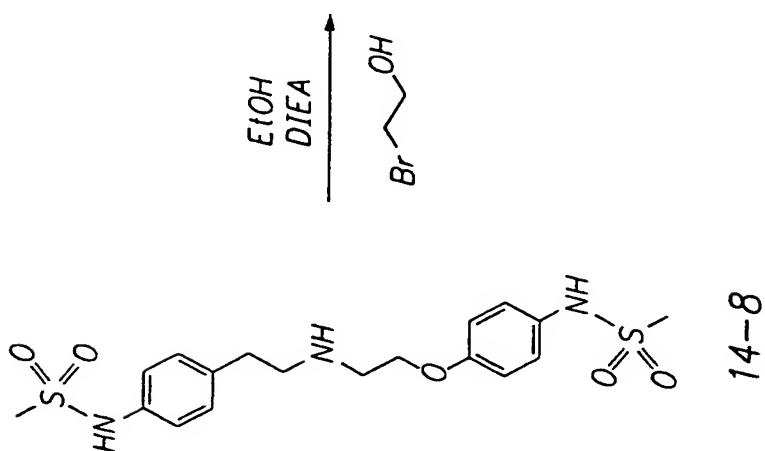
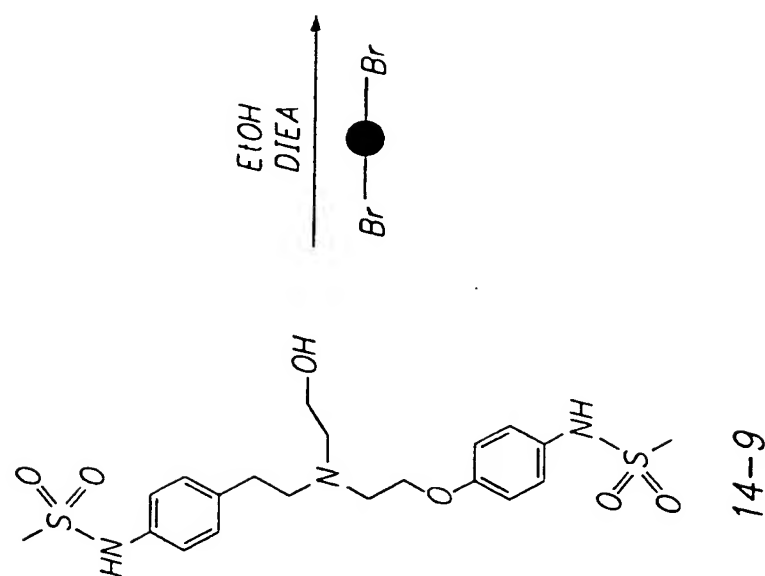
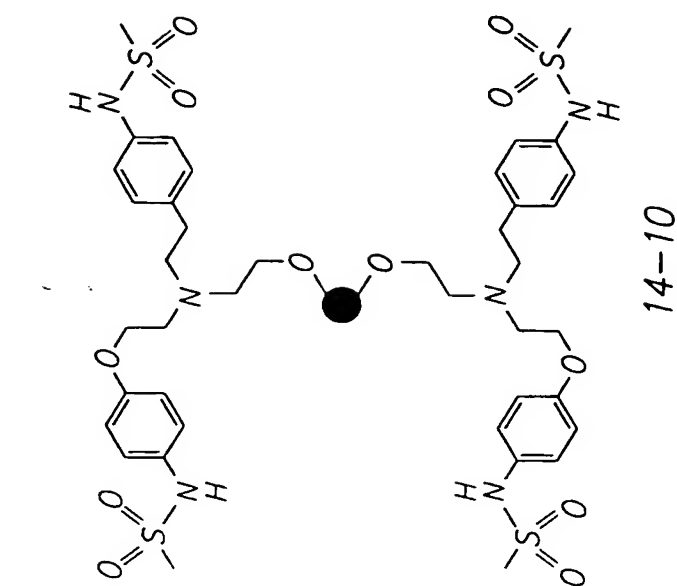


14-6

14-7



15/23

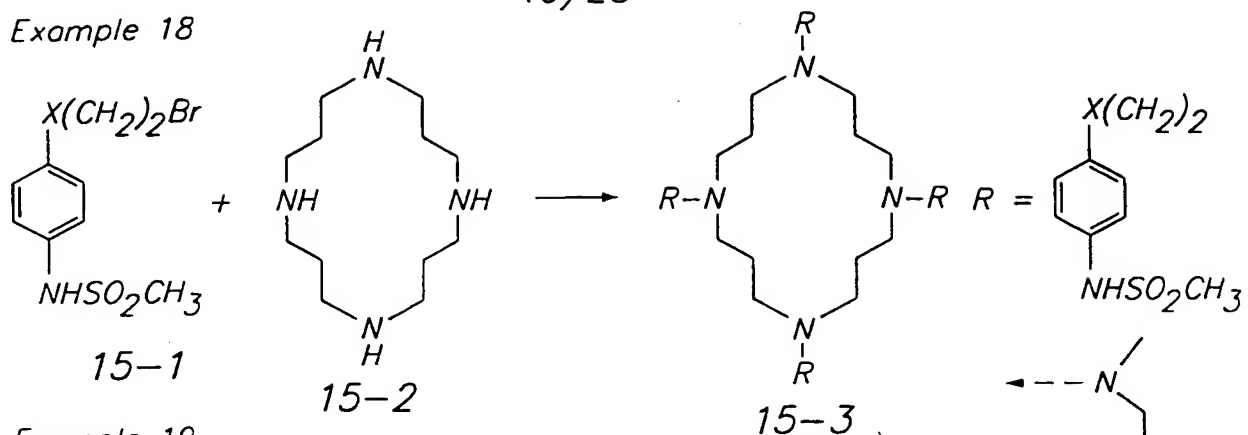


Introduction of Spacer to Facilitate Multivalomer Formation

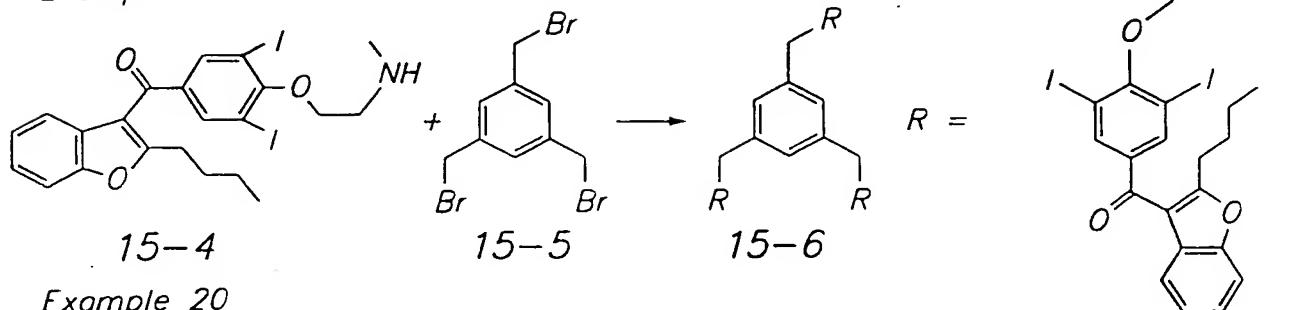
FIG. 14B

16/23

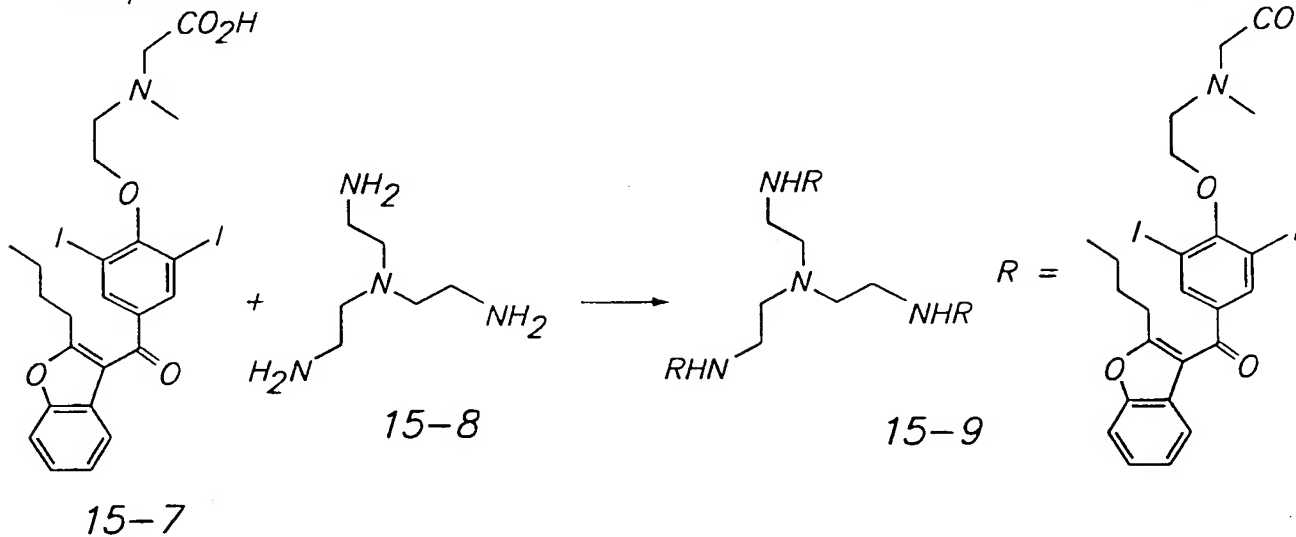
Example 18



Example 19



Example 20



Example 21

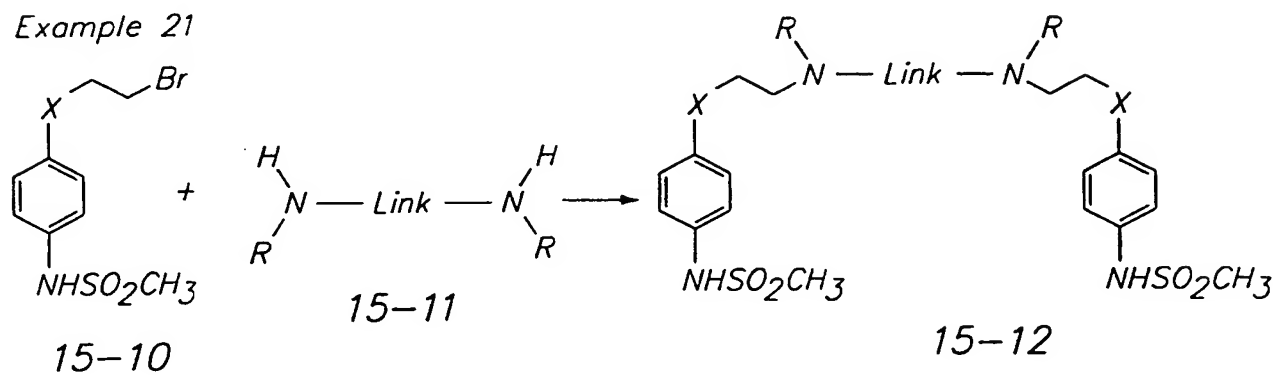


FIG. 15

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17/23

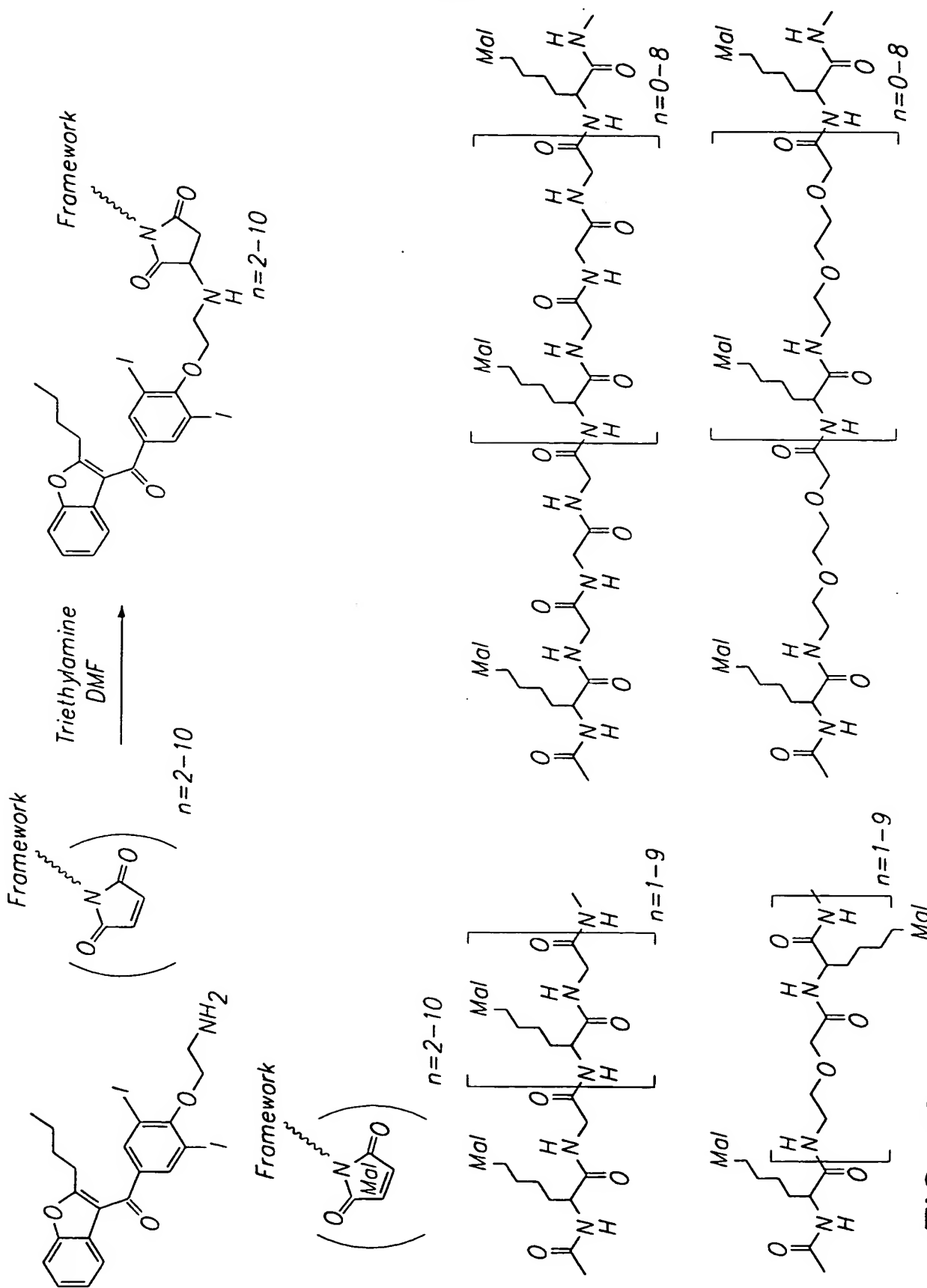
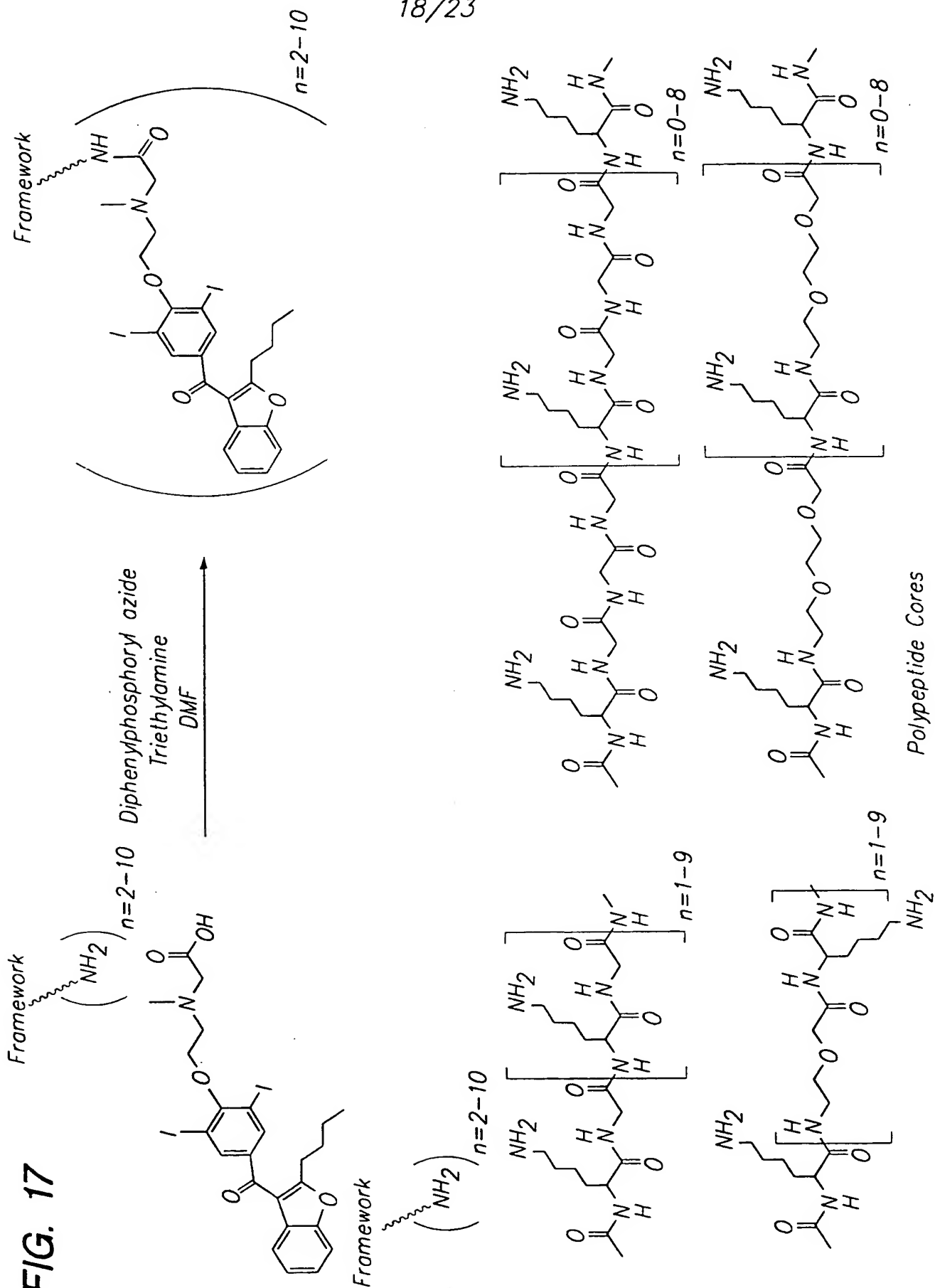


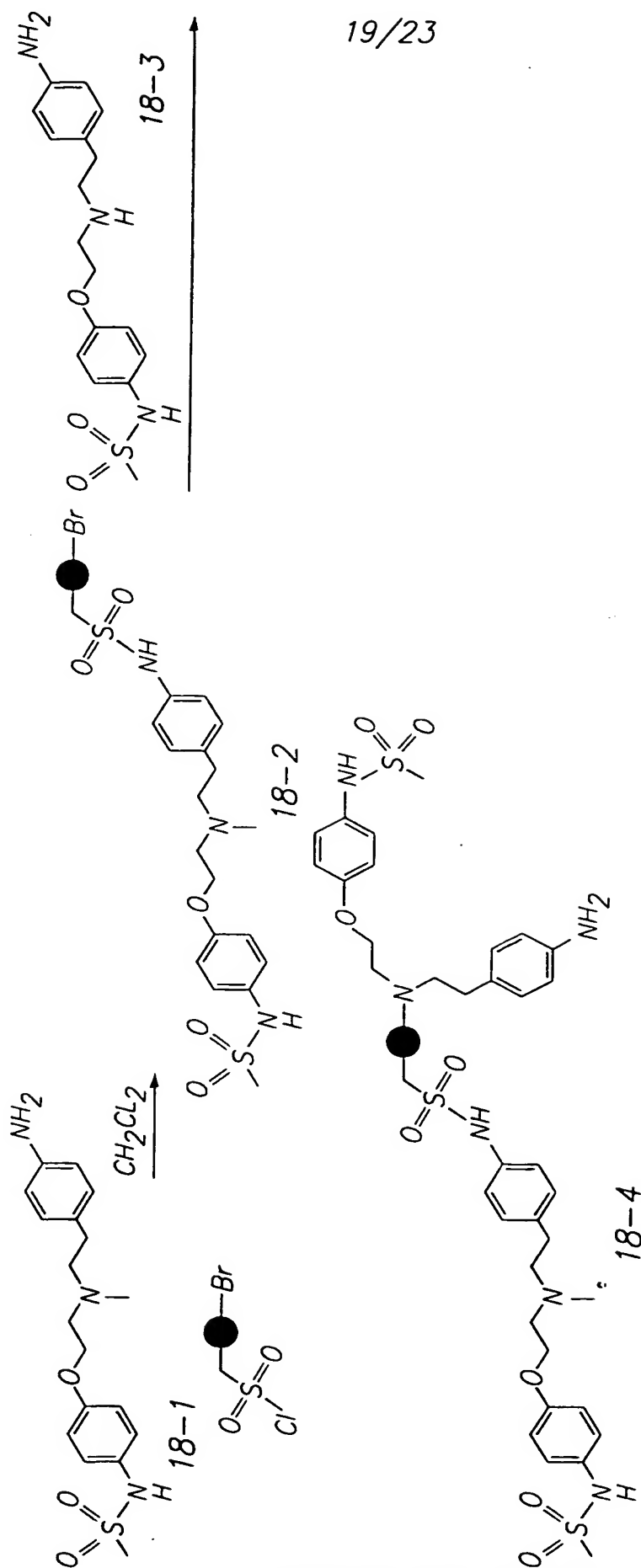
FIG. 16

Multimers with Peptide Core and Maleimide Coupling

18/23

FIG. 17



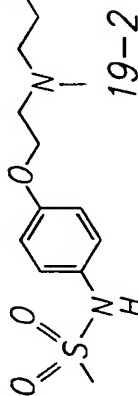
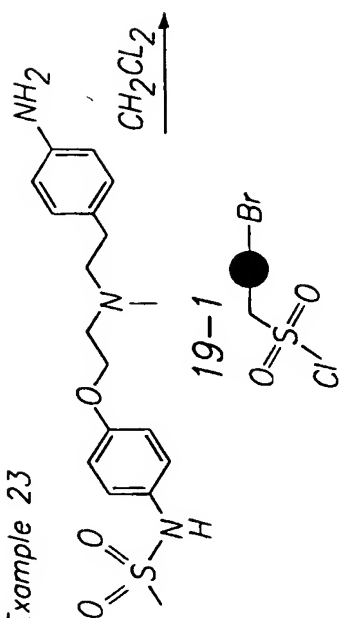


**FIG. 18** Asymmetric Linear Bivalent Compound

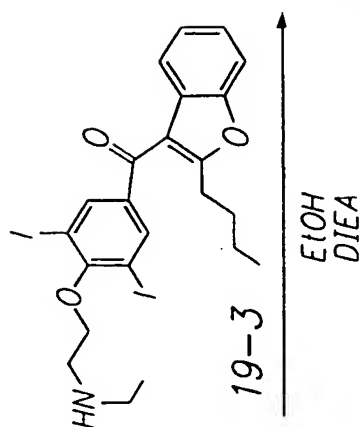
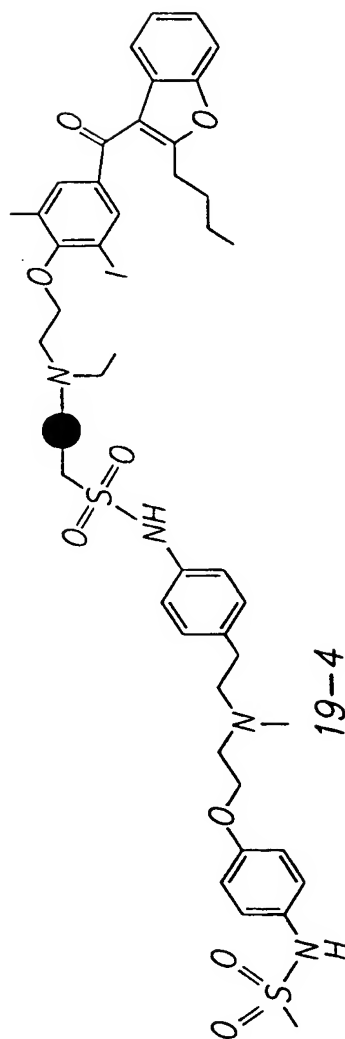
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FIG. 19A

Example 23



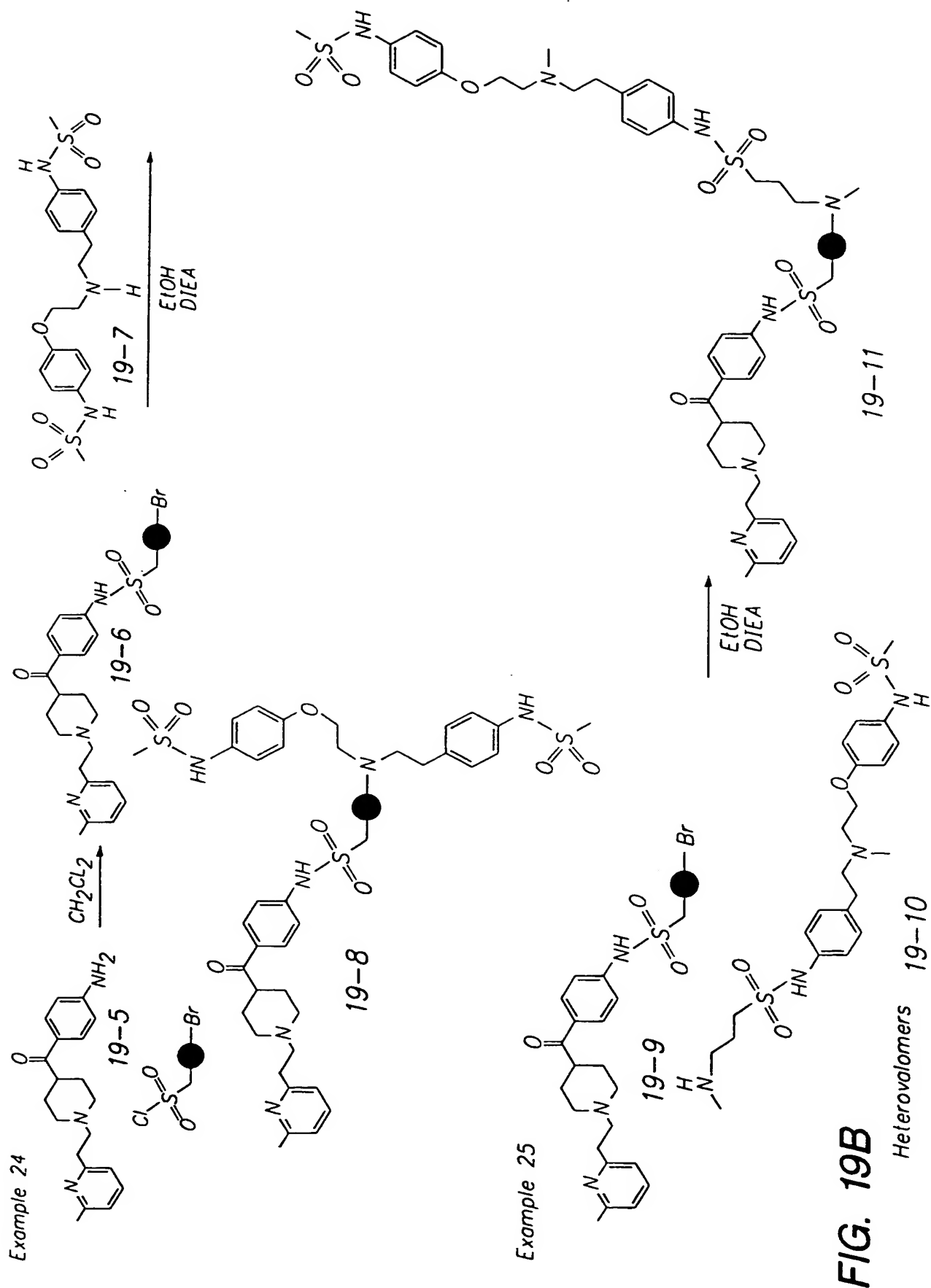
20/23

EtOH  
DIEA

Heterovalomers

SUBSTITUTE SHEET (RULE 26)

21/23



22/23

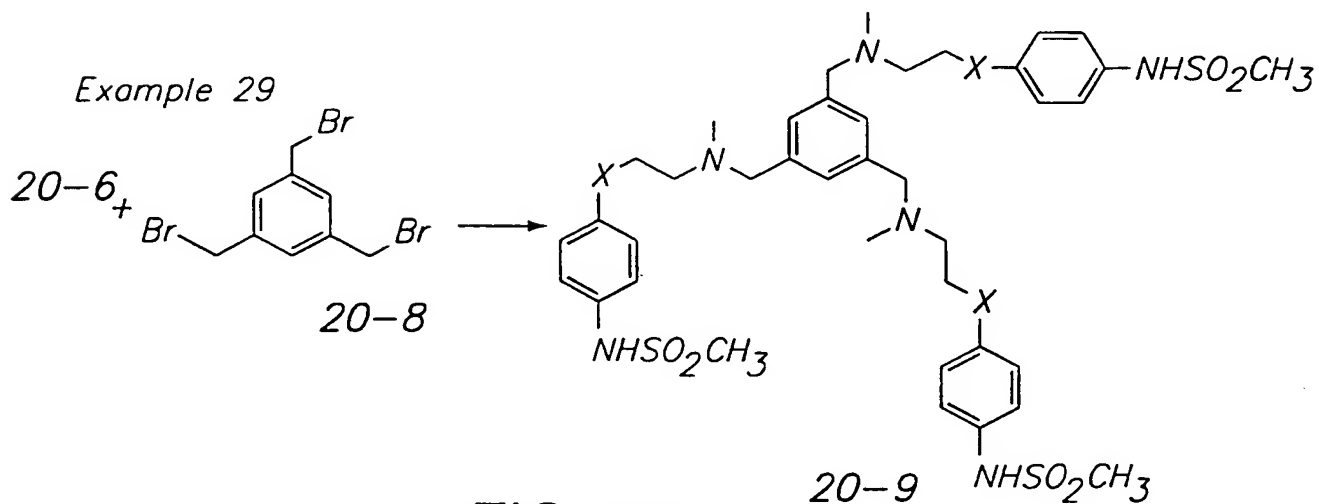
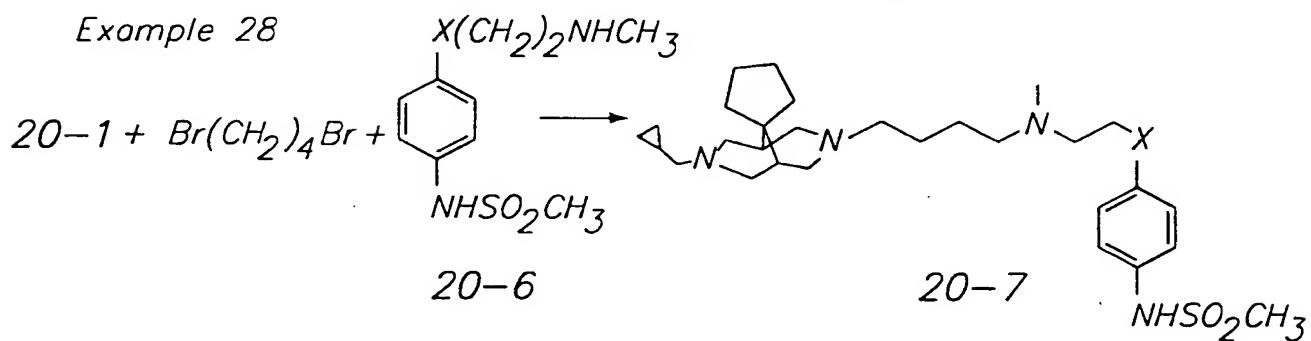
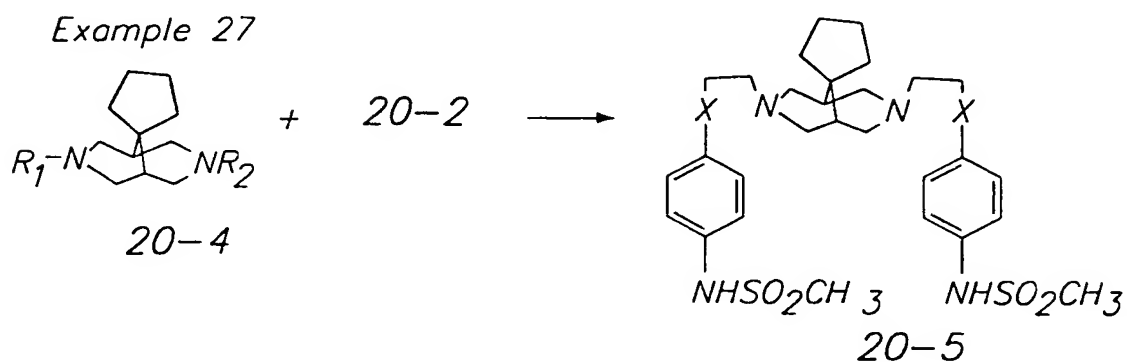
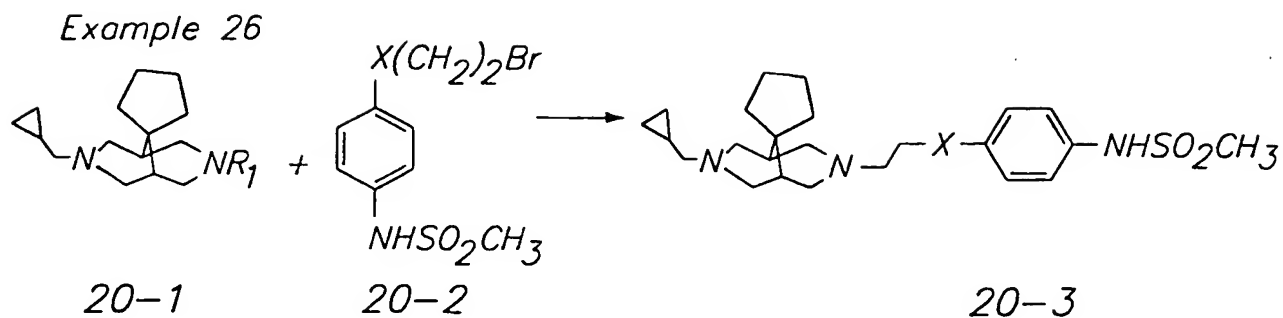
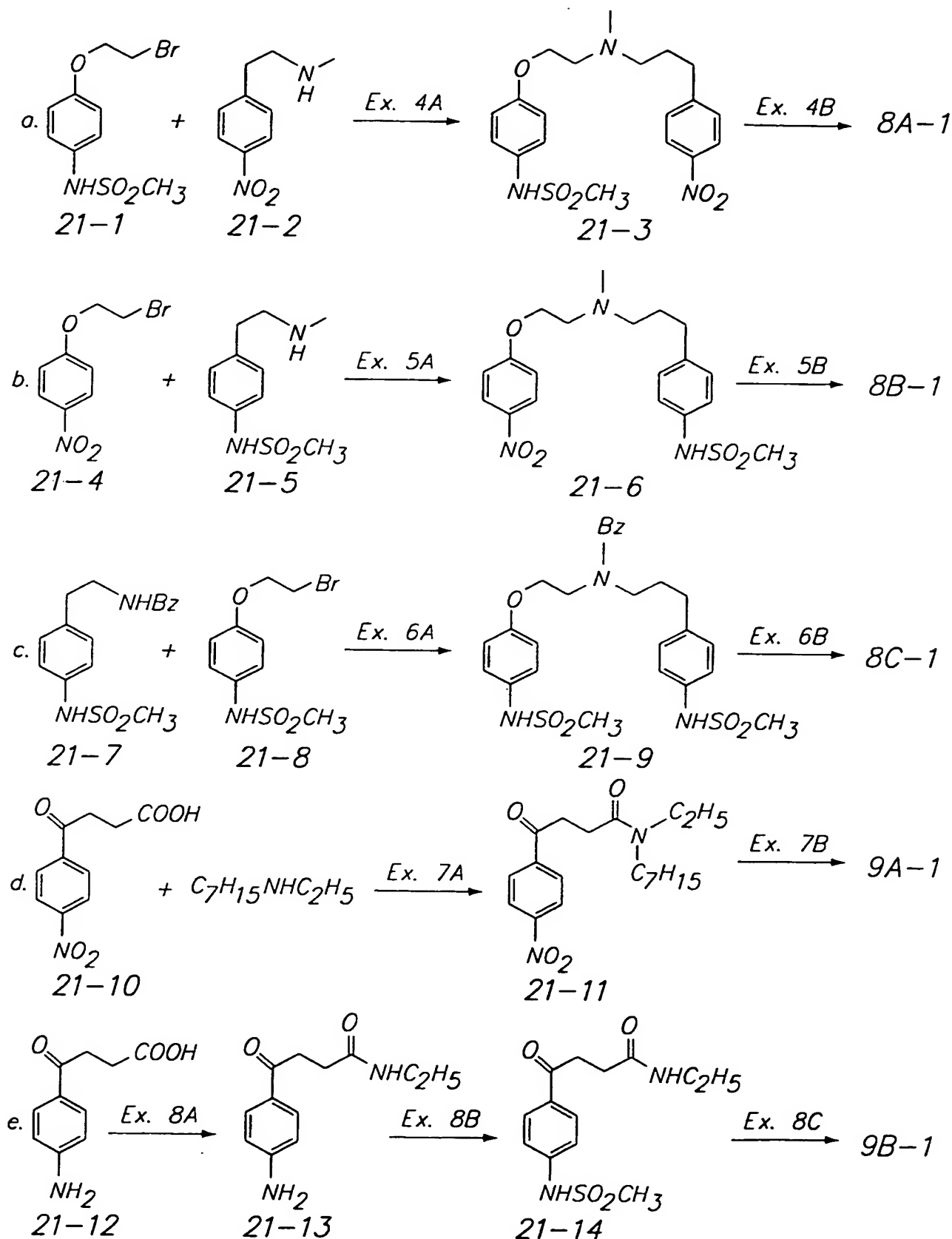


FIG. 20



23/23



Preparation of Intermediates

FIG. 21

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/12777

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN (CAPLUS, BIOSIS, EMBASE, MEDLINE, SCISEARCH)

Search Terms: potassium channel, multivalent, combinatorial, benzodia?

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/05802 A1 (NEORX CORPORATION) 16 April 1992 (16.04.92), see Abstract, page 3 lines 1-25, page 4 lines 20-27, page 5 lines 6-18, page 21 lines 4-33, page 22 lines 1-8 and claim 1.	1-49
Y	US 5,545,568 A (ELLMAN) 13 August 1996, see column 1 lines 21-53, column 6 lines 55-67 and column 7, lines 1-30.	1-49
Y	BUNIN et al. 'Synthesis and Evaluation of Three 1,4-Benzodiazepine Libraries.' In: Combinatorial Peptide and Nonpeptide Libraries, Edited by Gunther Jung, New York: VCH 1996, pages 405-424. See entire article, especially Introduction, page 405.	1-49

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

11 AUGUST 1999

Date of mailing of the international search report

22 OCT 1999

Name and mailing address of the ISA/US  
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PARALEGAL SPECIALIST  
CHEMICAL MATRIX

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/12777

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	SALATA et al. A Novel Benzodiazepine that Activates Cardiac Slow Delayed Rectifier K <sup>+</sup> Currents. Molecular Pharmacology. July 1998, Vol. 53, pages 220-230. See entire article.	1-49
Y	SHUKER et al. Discovering High-Affinity Ligands for Proteins: SAR by NMR. Science. 29 November 1996, Vol. 274, pages 1531-1534. See entire article, especially Figure 1.	23-49

Form PCT/ISA/210 (continuation of second sheet)(July 1992)\*

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/12777

A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (6):

A61K 38/00, 39/00, 39/44, 39/395, 51/00; C07K 2/00, 4/00; G01N 33/53, 33/543, 33/566

A. CLASSIFICATION OF SUBJECT MATTER:  
US CL :

424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807